МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА БІОТЕХНОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової кафедри
_____М. М. Барановський
«___»_____2021 р

ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ЗДОБУВАЧА ВИЩОЇ ОСВІТИ ОСВІТНЬОГО СТУПЕНЯ «БАКАЛАВР»
СПЕЦІАЛЬНІСТЬ 162 «БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ»
ОСВІТНЬО-ПРОФЕСІЙНА ПРОГРАМА «ФАРМАЦЕВТИЧНА БІОТЕХНОЛОГІЯ»

Тема: «Дослідження умов визначення 3,6-дихлоро-2-метоксибензойної кислоти»

Виконавець: студентка 404гр.ФЕБІТ Кушнірук А.В.

Керівник: к.х.н, доцент Мага І.М.

Нормоконтролер: Дражнікова А.В.

КИЇВ 2021

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL AVIATION UNIVERSITY
FACULTY OF ENVIRONMENTAL SAFETY, ENGINEERING AND TECHNOLOGIES
DEPARTMENT OF BIOTECHNOLOGY

ACCEPT TO DEFENSE
Head of the Department
_____M. M. Baranovsky
«____» _____ 2021

BACHELOR THESIS

(EXPLANATORY NOTE)

APPLICANT OF HIGHER EDUCATION OF EDUCATIONAL DEGREE BACHELOR

SPECIALTY 162 "BIOTECHNOLOGY AND BIOENGINEERING"
EDUCATION- PROFESSIONAL PROGRAM "PHARMACEUTICAL BIOTECHNOLOGY"

Theme: «Investigation of conditions for determination of 3,6-dichloro-2-methoxybenzoic acid »

Executor: student of group 404.FESET Kushniruk A.V.

Supervisor of studies: PhD in Chemistry, Assoc. Prof Maga I.M.

Supervisor of normal inspection: Drazhnikova A.V.

KYIV 2021

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

Факультет екологічної безпеки інженерії та технологій

Кафедра біотехнології

Спеціальність 162 «Біотехнології та біоінженерія»

Освітньо - професійна програма « Фармацевтична біотехнологія»

ЗАТВЕРДЖУЮ

Завідувач кафедри

_____М.М.Барановський

«___»_____2021 р.

ЗАВДАННЯ

на виконання дипломної роботи

Кушнірук Анни Вікторівни

1.Тема дипломної роботи: «Дослідження умов визначення 3,6-дихлоро-2-метоксибензойної кислоти» затверджена наказом ректора від «___» _____ 2021 р. №_____

2. Термін виконання роботи: з 26 квітня по 08 червня 2021 р.

3. Вихідні дані роботи: Літературні джерела, щодо будови та фізико-хімічних властивостей 3,6-дихлоро-2-метоксибензойної кислоти (дікамби). ЇЇ токсичності, методів синтезу. Наукова інформація, щодо вилучення та методів аналізу дікамби.

4. Зміст пояснювальної записки: ВСТУП; РОЗДІЛ 1 ХАРАКТЕРИСТИКА 3,6-ДИХЛОРО-2-МЕТОКСИБЕНЗОЙНОЇ КИСЛОТИ ТА ЇЇ ЗАСТОСУВАННЯ ЯК АКТИВНОЇ РЕЧОВИНИ ГЕРБИЦИДІВ; РОЗДІЛ 2. ХАРАКТЕРИСТИКА ТОКСИЧНОСТІ 3,6-ДИХЛОР-2-МЕТОКСИБЕНЗОЙНОЇ КИСЛОТИ ТА ГЕРБІЦИДІВ, ДО ЯКИХ ВОНА ВХОДИТЬ; РОЗДІЛ 3. МЕТОДИ ОДЕРЖАННЯ 3,6-

ДИХЛОРО-2-МЕТОКСИБЕНЗОЙНОЇ КИСЛОТИ. РОЗДІЛ 4 ПРОБОПІДГОТОВКА ТА МЕТОДИ ВИЛУЧЕННЯ 3,6-ДИХЛОРО-2-МЕТОКСИБЕНЗОЙНОЇ КИСЛОТИ; РОЗДІЛ 5. МЕТОДИ ВИЗНАЧЕННЯ 3,6-ДИХЛОРО-2-МЕТОКСИБЕНЗОЙНОЇ КИСЛОТИ; ВИСНОВКИ; ЛІТЕРАТУРА.

5. Перелік обов'язкового графічного (ілюстративного) матеріалу: 21 рисунок.

6. Календарний план-графік.

№	Завдання	Термін виконання
1	Вибір теми дипломної роботи, узгодження змісту з дипломним керівником	26.04.2021
2	Складання схеми виконання бакалаврської дипломної роботи.	27.04.2021
3	Підбір та обробка літератури по характеристиці 3,6-дихлор-2-метоксибензойної кислоти та її застосуванню як діючої речовини гербіцидних препаратів.	28.04.2021 – 02.05.2021
4	Підбір та обробка літератури по токсичності 3,6-дихлор-2-метоксибензойної кислоти та гербіцидних препаратів до яких вона входить.	03.05.2021 – 04.05.2021
5	Підбір та обробка літератури по методам добування3,6-дихлор-2-метоксибензойної кислоти.	05.05.2021 – 08.05.2021
6	Підбір та обробка літератури по методам вилучення 3,6-дихлор-2-метоксибензойної кислоти	09.05.2021 – 16.05.2021
7	Підбір та обробка літератури по спектральним та хроматографічним методам аналізу 3,6-дихлор-2-метоксибензойної кислоти	17.05.2021 – 24.05.21021

8	Оформлення дипломної роботи.	24.05.2021 – 29.05.2021
9	Перевірка дипломної роботи керівником.	30.05.2021
10	Коригування, підготовка доповіді та презентації	31.05.2021 -01.06.2021
11	Передзахист дипломної роботи.	02.06.2021
12	Захист дипломної роботи	06.2021

7. Дата видачі завдання: «26» квітня 2021 р.

Керівник дипломної роботи_____ Мага І. М.

(підпис керівника)

Завдання прийняла до виконання _____ Кушнірук А. В.

(підпис виконавця)

NATIONAL AVIATION UNIVERSITY

Faculty of environmental safety, engineering and technologies

Department of Biotechnology

Specialty 162 “Biotechnology and bioengineering”

Education program “Pharmaceutical biotechnology”

APPROVED

Head of the Department

_____M.M.Baranovsky

«___» _____ 2021

TASK

FROM BACHELOR THESIS OF STUDENT

Kushniruk Anna Viktorivna

1.The theme of the thesis: « Investigation of conditions for determination of 3,6-dichloro-2-methoxybenzoic acid » approved by the order of the rector from "__" _____ 2021, No. _____

2. The term of the work: from 26 April till June 08, 2021.

3. Output data: Literature sources on the structure and physicochemical properties of 3,6-dichloro-2-methoxybenzoic acid (dicamba). its toxicity, methods of synthesis. Scientific information on dicamba extraction and analysis methods.

4. Contents of the explanatory note: INTRODUCTION; CHAPTER 1. CHARACTERISTICS OF 3,6-DICHLORO-2-METHOXYBENZOIC ACID AND ITS USE AS AN ACTIVE SUBSTANCE OF HERBICIDES; CHAPTER 2. TOXICITY CHARACTERISTICS OF 3,6-DICHLORO-2-METHOXYBENZOIC ACID AND HERBICIDES WHICH IT INCLUDES; CHAPTER 3. METHODS OF PREPARATION

3,6-DICHLORO-2-METHOXYBENZOIC ACID; CHAPTER 4. SAMPLE PREPARATION AND DICAMBA EXTRACTION; CHAPTER 5. METHODS DETERMINATION OF DICAMBA; CONCLUSIONS; REFERENCES:

5. List of compulsory graphic (illustrative) material: 21 figures.

6.Schedule.

№	Task	Execution term
1	Selection of the theme of the thesis, agreement of the content with the supervisor.	26.04.2021
2	Drawing up of the scheme of performance of the bachelor thesis.	27.04.2021
3	Selection and processing of literature on the characteristics of 3,6-dichloro-2-methoxybenzoic acid and its use as an active ingredient in herbicides.	28.04.2021 – 02.05.2021
4	Selection and processing of literature on the toxicity of 3,6-dichloro-2-methoxybenzoic acid and herbicides, which it includes.	03.05.2021 – 04.05.2021
5	Selection and processing of literature on methods of obtaining 3,6-dichloro-2-methoxybenzoic acid.	05.05.2021 – 08.05.2021
6	Selection and processing of literature on the methods of extraction of 3,6-dichloro-2-methoxybenzoic acid	09.05.2021 – 16.05.2021
7	Selection and processing of literature on spectral and chromatographic methods of analysis of 3,6-dichloro-2-methoxybenzoic acid	17.05.2021 – 24.05.21021
8	Writing the thesis.	24.05.2021 – 29.05.2021

9	Examination of the thesis by the supervisor.	30.05.2021
10	Editing of the speech and final presentation	31.05.2021 -01.06.2021
11	Preliminary defence of graduating work.	02.06.2021
12	Defence of graduating work	06.2021

7. Date of task receiving: «12» May 2021

Supervisor of bachelor thesis _____ Maga I.M.

(sign of supervisor)

Task for execution was taken by _____ Kushniruk A.V.

(sign of graduating student)

РЕФЕРАТ

Пояснювальна записка до дипломної роботи «Дослідження умов визначення 3,6-дихлоро-2-метоксибензойної кислоти»: 49 сторінок, 21 рисунок, 24 використаних джерел.

Об'єкт дослідження – Дослідження методів визначення та вилучення 3,6-дихлоро-2-метоксибензойної кислоти. Важливість розробки методів визначення дикамби.

Мета дипломної роботи – дослідження умов визначення 3,6-дихлор-2-метоксибензойної кислоти спектроскопічними та хроматографічними методами та способи вилучення її з матриці.

Методи дослідження – аналітичні, синтетичні, математичні, порівняння.

Предмет дослідження – 3,6-дихлор-2-метоксибензойна кислота, гербіциди містять дікамбу, рослинні продукти, екологічні об’єкти, способи вилучення, методики аналізу.

Ключові слова: 3,6-ДИХЛОР-2-МЕТОКСИБЕНЗОЙНА КИСЛОТА, ТОКСИЧНІСТЬ, МЕТОДИ ДОБУВАННЯ, ЕКСТРАКЦІЯ, СПЕКТРОФОТОМЕТРІЯ, РАМАН СПЕКТРОСКОПІЯ, ВЕРХ, МЕТОДИКА ВИЗНАЧЕННЯ.

ABSTRACT

Explanatory note to the thesis « Investigation of conditions for determination of 3,6-dichloro-2-methoxybenzoic acid », 49 pages, 21 figures, 24 sources used.

Object of investigation: Investigation of methods for determination and extraction of 3,6-dichloro-2-methoxybenzoic acid. The importance of developing methods for determining dicamba.

Purpose of the work - study of conditions for determination of

3,6-dichloro-2-methoxybenzoic acid by spectroscopic and chromatographic methods and way of its extraction from the matrix.

Methods of research – Analytical, synthetic, mathematical, comparative.

Subject of investigations: 3,6-dichloro-2-methoxybenzoic acid, herbicides contain dicamba, plant products, ecological objects, methods of extraction, methods of analysis.

Key words: 3,6-DICHLORO-2-METHOXYBENZOIC ACID, TOXICITY, PREPARATION METHODS, EXTRACTION, SPECTROPHOTOMETRY, RAMAN SPECTROSCOPY, HPLC, METHOD OF DETERMINATION.

CONTENT

INTRODUCTION

Actuality. 3,6-Dichloro-2-methoxybenzoic acid (dicamba) is an important and selective systemic herbicide. It is effective against annual and perennial broad-leaved weeds and brushed species in cereals, maize, sorghum, sugarcane, turf, pastures, range land and non-crop areas. Dicamba is absorbed through roots as well as leaves and translocates throughout the plant. It mimics auxin, a plant growth regulator and at adequate concentrations, is known to increase plant growth rate that outgrows its nutrient supplies leading to death of the plant. Dicamba in combination with phenoxy or other herbicides is used in pastures, range land, and non-crop areas to control weeds.

The purpose of the work is investigation of the conditions for determination of 3,6-dichloro-2-methoxybenzoic acid and the conditions for its extraction from the matrix.

- To achieve this goal, the following tasks were set:

1.Analyze the status and properties of 3,6-dichloro-2-methoxybenzoic acid and its properties as a compound and as an active ingredient in herbicides.

2.Determine the toxic characteristics of dicamba.

3.Investigate the schemes of dicamba synthesis.

4.Establish methods for extracting dicamba from the studied objects.

5.Investigate recovery techniques and methods for determining dicamba.

Object of investigation: investigation of methods for the determination and extraction of 3,6-dichloro-2-methoxybenzoic acid. The importance of dicamba determination.

Subject of investigations: 3,6-dichloro-2-methoxybenzoic acid, Herbicide preparations containing dicamba, Food of plant origin, Environmental objects.

Research methods: analytical, synthetic, mathematical, comparative.

Scientific novelty of the results. New theoretical studies of the reserches and properties of 3,6-dichloro-2-methoxybenzoic acid, herbicidal activity, toxicity, extraction methods, isolation methods, as well as various methods of analysis of dicamba.

The practical significance of the results. Descriptive important methods of extraction, synthesis of 3,6-dichloro-2-methoxybenzoic acid, methods of its isolation from different matrices, sensitive methods for determining dicamba by spectroscopic and chromatographic methods, which may form the basis for the development of new highly sensitive methods of analysis, 6-dichloro-2- methoxybenzoic acid.

Graduate's personal contribution. The entire volume of theoretical research on the topic of the thesis, analysis of literature data, description and processing of the analyzed materials were performed by the graduate personally under the guidance of Ph.D., Associate Professor Maga I.M.

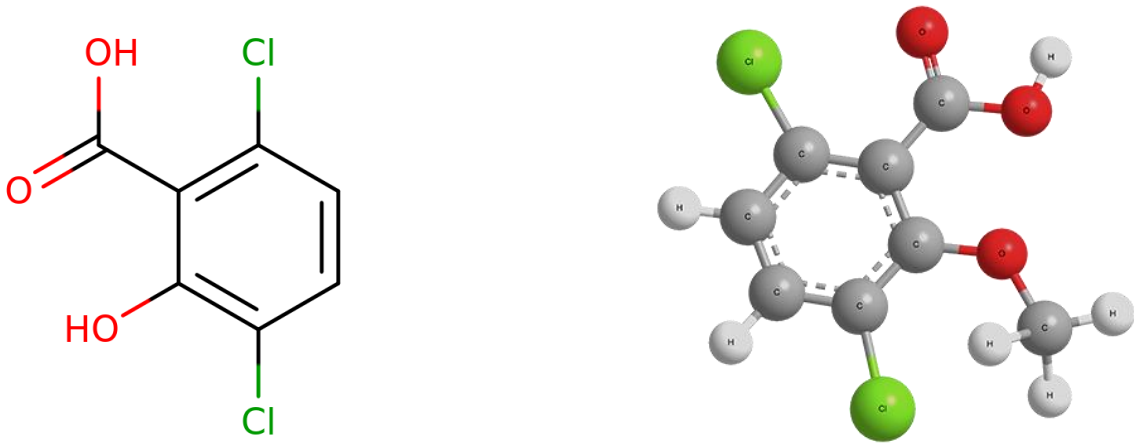
CHAPTER 1

CHARACTERISTICS OF 3,6-DICHLORO-2-METHOXYBENZOIC ACID AND ITS USE AS AN ACTIVE SUBSTANCE OF HERBICIDES

Pesticides are widely used in various stages of cultivation and to protect products from pests postharvest. Furthermore, they preserve the quality of the products. In spite of the positive effects of the application of pesticides in agriculture, many pesticides are harmful to the environment and are known or suspected to be toxic to humans [1]. Their adverse effects on human health may include acute neurologic toxicity, chronic neurodevelopment impairment, cancer, and endocrine dysfunction. Monitoring pesticide residues in food is of great importance to ensuring food safety. Herbicides are used to control weeds, among which are formulations with the active ingredient 3,6-dichloro-2-methoxybenzoic acid, which has the technical name dicamba.

1.1. Characteristics of 3,6-dichloro-2-methoxybenzoic acid and its use as an active substance of herbicides

Dicamba has preferred IUPAC name 3,6-dichloro-2-methoxybenzoic acid (Fig. 1.1). Other names 3,6-Dichloro-o-anisic acid, Dianat. It is a synthetic auxin and is used as a herbicide. According to the chemical classification, it is also referred to as chlorinated derivative of o-anisic acid. Density1.57 g/dm^3, melting point 114 to 116 °C, Flash point 199 °C. Solubility (20oC, g/L): in water 500; in acetone 810. in ethanol 922, in hexane 2,800, methanol 500, ethyl acetate 500, cyclohexanone – 916, dioxane - 1180, toluene - 130, xylene -78, dichloromethane - 260 [2]. Brand names for formulations of this herbicide include Dianat, Banvel, Diablo, Oracle and Vanquish etc [3].



a *b*

Fig. 1.1. Chemical structural formula of dicamba (*a*) and patial ball-rod model (*b*)

1.2. Physical and chemical properties of dicamba

Dicamba is white crystalline substance. Well soluble in organic solvents, poorly in water. Resistant to acids and alkalis. Technical preparation (83 and 87% of d.r.) is a golden-brown crystalline powder. Contains 13-17% of impurities with a predominance of 2-methoxy-3,5-dichlorobenzoic acid. Dicamba salts with alkali metals and organic bases are well soluble in water [4]. Dicamba in an aqueous medium dissociates quite well with the formation of a hydrogen cation and a dicamba anion (Fig. 1.2). Dicamba has a low organic carbon partition coefficient and therefore, a low affinity for soil particles and suspended sediment. Dicamba has a low octanol/water partition coefficient and is resistant to oxidation and hydrolysis under most conditions.

The dissociation constant is pKa = 1.87, then it manifests itself as a weak acid. However, it is a stronger acid in comparison with benzoic acid (pKa = 4.20) due to the presence of two chlorine - electron-acceptor substituents in the aromatic nucleus.

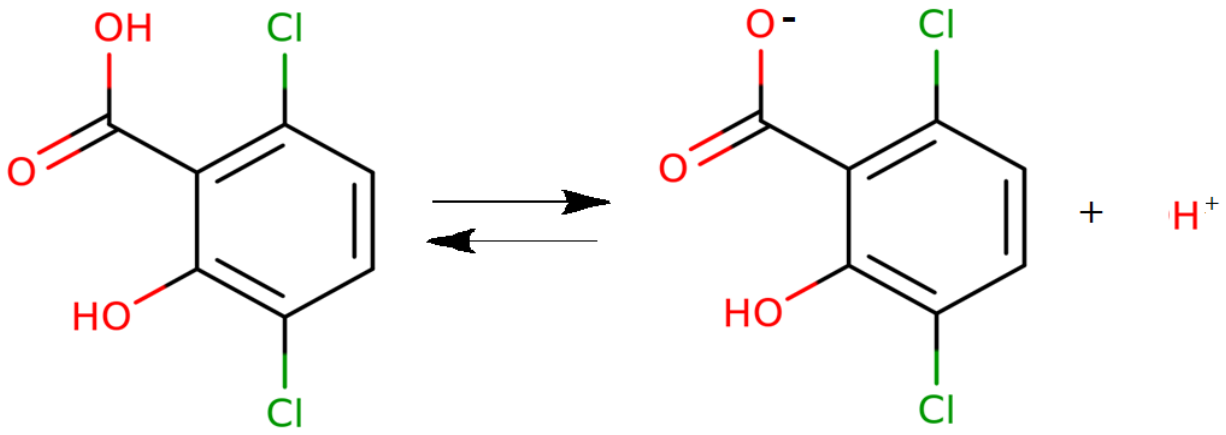
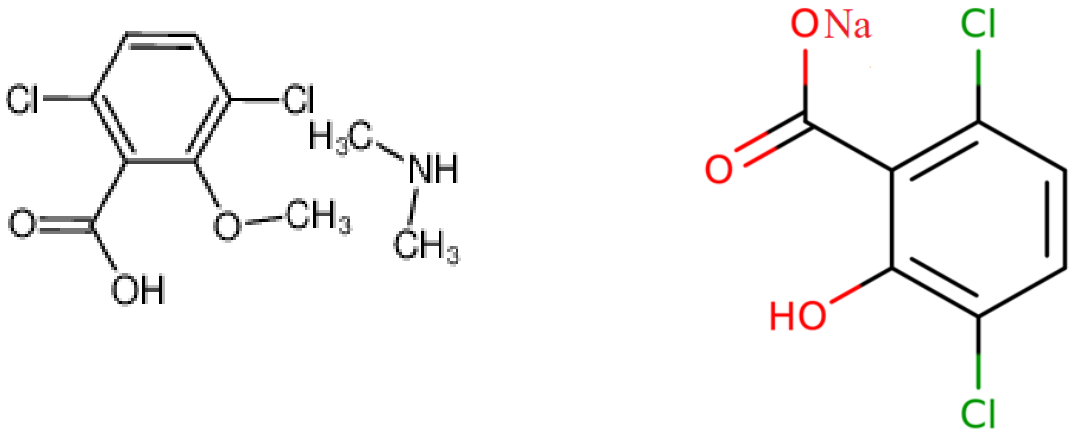


Fig. 1.2. Equation of dissociation of dicamba.

1.3. Herbicidal activity of dicamba

Formulations include dicamba acid, dimethylamine salt, sodium salt, diglycoamine salt, isopropylamine salts, and potassium salt. Dicamba is often used in the form of dimethylamine or sodium salt, the chemical formulas of which are shown in Fig. 1.3.



a *b*

Fig. 1.3. The chemical formula of dicamba in the form of dimethylamine salt (*a*) and sodium salt (*b*).

1.4. Crops and weeds for which dicamba is used

Some signs and symptoms from a brief exposure to dicamba. Pure dicamba is low in toxicity if breathed. If inhaled, people may experience dizziness, and irritation of the nose, resulting in coughing. If you get pure dicamba on your skin, it is low in toxicity, however skin irritation may develop. If you get dicamba in your eyes, it is moderately toxic. If dicamba is swallowed, people have reported symptoms such as vomiting, loss of appetite and muscle spasms. If a large amount is swallowed, diarrhea and abdominal pain have been reported [6]. Pets may be exposed to dicamba if they come into contact with plants that have been treated with dicamba, either by eating the plants or walking through an area where dicamba was applied. Signs that a dog or a cat may have been exposed to dicamba include shortness of breath, muscle spasms and the animal may produce a lot of saliva [7]. Birds may also be exposed to dicamba by eating dicamba granules and signs include wing drop, a loss of controlled movements, and weakness.

1.5. The mechanism of action of dicamba on weeds

Dikamba - herbicide of leaf and soil action. Manufactured by a large number of companies that specialize in pesticides. As an example, Figure 1.4 shows samples of a commercial preparation of dicamba from “OzCrop” (Australia) and “Alfa Smart Agro” (Ukraine) in the form of five liter canisters. Dicamba has since been used for household and commercial weed control.

The herbicide suppresses the following species: asterisk, creeping mustard, field mustard, mountain ash and sprawling, yarrow, butterbur and other perennial weeds in non-agricultural lands (in this case, the toxicity of the drug in the soil persists for up to 2 years). In smaller doses, the herbicide is used for spraying vegetative weeds (buttercup, hellebore,

borscht, sorrel) in the spring on hayfields and in autumn on pastures. The drug in higher doses affects perennial weeds: creeping bitter gourd, birch, ragweed, thistle and other plants, so they can be sprayed in distribution centers, as well as on pastures and meadows, in areas without cultivated plants. Preparations are recommended for spraying crops of winter rye, wheat, oats, barley (0.15–0.5 l / ha), millet (0.4–0.5 l / ha) in the tillering phase of the crop and 2–4 leaves in annuals and 15 cm in height for perennial weeds. Sowings of corn (0.4–0.8 l / ha) are sprayed in the phase of 3-5 leaves of the crop and at 15 cm in height for perennial weeds [8].

a *b*

Fig.1. 4. Samples of a commercial preparation of dicamba from: (*a*) “OzCrop” (Australia) and (*b*) “Alfa Smart Agro” (Ukraine).

The herbicidal activity of dicamba is similar to 2,3,6-trichlorobenzoic acid, but slightly superior to it in potency. Dicamba can be classified as a herbicide with auxin-like activity. Its effect is manifested in increasing the rate of RNA synthesis and its concentration, accelerating the synthesis of lipids and protein, increasing the extensibility of membranes and cell growth in length. Dicamba is characterized by mobility in plants. Penetrating through the leaves into plants, it moves quickly into the root system. Moves along the phloem and xylem, accumulating mainly in the growing tips. From the roots of a small amount of the drug can pass into the environment. When processing the roots do not accumulate in them, and moves to the upper parts of the plant. The redistribution of the herbicide from mature leaves and its

concentration in the young is possible, from where its transfer is significantly slowed down. In drug-resistant cereals, the herbicide, evenly distributed throughout the plant, is destroyed fairly quickly. Most of it is released into the environment from the root system. Cereals in the tillering phase are insensitive to this compound. External signs of dicamba damage include elongation of the stem, twisting and wilting of the leaves, and then their death.

On the Figure 1.5 shows the effects of dicamba herbicide application on agricultural lands from Moncanto (USA) (a, b) and Singenta (Switzerland) (c, d). Pictures a) and (c) the control area of the field from the firms “Moncanto”: and “Singenta” respectively is not treated with herbicides, Pictures (b) and (d) area of the field treated with dicamba herbicides from “Moncanto”: and “Singenta”, respectively. It can be seen from both figures that in the fields cultivated with dicamba (b, d) only cultivated plants grow and there are practically no weeds. That is, cultivated plants have no competition for nutrients and light, and they grow more intensively and more fertile.

a

b

c

d

Fig. 1.5. The effects of dicamba herbicide application on agricultural land “Monsanto” (USA) (*a*, *b*) and “Singenta” (Switzerland) (*c*, *d*). Figures a) and (*c*) the control plot of the field from the firms “Monsanto”: and “Singenta”, respectively, not treated with herbicides; (*b*) and (*d*) images of a field treated with dicamba herbicides from Moncanto: and Singenta, respectively.

1.6. Conclusions to the chapter

3,6-dichloro-2-methoxybenzoic acid (dicamba) belongs to the synthetic auxin class and exhibits herbicidal activity. Chemically, dicamba is a weak organic acid. The most commonly used forms of herbicides are dimethylamine and sodium salt. Its effect is manifested in increasing the rate of RNA synthesis and its concentration, accelerating the synthesis of lipids and protein, increasing the extensibility of membranes and cell growth in length.

CHAPTER 2
TOXICITY CHARACTERISTICS OF 3,6-DICHLORO-2-METHOXYBENZOIC ACID AND HERBICIDES WHICH IT INCLUDES

2.1. Environmental impact of dicamba use

When using dicamba as a herbicide, only a small part of the substance gets on the plant and has an herbicides effect. Most of the drug is washed into the soil and stays there for a long time, gradually decomposing and metabolized by microorganisms. While in the soil, it has a detrimental effect on the positive microflora. Surface water and groundwater are also washed away from the soil. In addition, the amount of dicamba that gets on the plants can be absorbed by them and then get into the fruits of plants unevenly distributed inside the plant in residual amounts of 3,6-dichloro-2-methoxybenzoic acid. Half of the dicamba trapped in the soil is removed from one and a half weeks to one and a half months. Given the acidic nature of dicamba, it is more retained in acidic soils, ie at a lower value of the acidity of the medium. Geological studies of different countries record dicamba in groundwater at the level of 10-2 mg / l. Moreover, such and more concentrations are recorded not only in areas of agricultural land, but also in non-agricultural and urban areas. In addition to dicamba esters, other compounds are highly hydrophilic and can migrate in aqueous solutions [9]. Moving in soil and water can harm other plants.

The presence of gaseous substances in the soil accelerates its transformation into a gas phase and transmission over long distances. When in contact with metals, dicamba will cause corrosion and gradual dissolution of the metal in the contact zone, causing gradual destruction of the structure. It is also toxic to algae and aquatic plants.

2.2. Toxicity to humans and warm-blooded animals

Some signs and symptoms from a brief exposure to dicamba. Pure dicamba is low in toxicity if breathed. If inhaled, people may experience dizziness, and irritation of the nose, resulting in coughing. If you get pure dicamba on your skin, it is low in toxicity, however skin irritation may develop. If you get dicamba in your eyes, it is moderately toxic. If dicamba is swallowed, people have reported symptoms such as vomiting, loss of appetite and muscle spasms. If a large amount is swallowed, diarrhea and abdominal pain have been reported [10].

Pets may be exposed to dicamba if they come into contact with plants that have been treated with dicamba, either by eating the plants or walking through an area where dicamba was applied. Signs that a dog or a cat may have been exposed to dicamba include shortness of breath, muscle spasms and the animal may produce a lot of saliva. Birds may also be exposed to dicamba by eating dicamba granules and signs include wing drop, a loss of controlled movements, and weakness.

2.3. Conclusions to the chapter

Dicamba is mobile in most soils and significant leaching is possible. The adsorption of dicamba to organo-clay soil is influenced by soil pH with the greatest adsorption to soil occurring in acidic soils. Dicamba is moderately persistent in soil. Its reported half-life in soil ranges from 1 to 6 weeks. Dicamba salts used in some herbicides are highly soluble in water. Therefore, from agricultural fields they can penetrate into surface and underground waters. Given the toxicity of dicamba, there is a need for constant monitoring of environmental objects for the presence of dicamba.

CHAPTER 3
METHODS OF PREPARATION 3,6-DICHLORO-2-METHOXYBENZOIC ACID

3.1. General characteristics of the synthesis of dicamba. Conversion of 2,5-dichloroaniline to 2,5-dichlophenol

A process for the preparation of 3,6-dichloro-2-methoxybenzoic acid (dicamba) involves diazotizing 2,5-dichloroaniline with nitrosylsulfuric acid in at least one first fluid medium at a temperature in the range of -15°C to 50 °C to obtain 2,5-dichlorophenyldiazonium salt; hydroxylating the 2,5- dichlorophenyldiazonium salt by contacting the 2,5-dichlorophenyldiazonium salt with sulfuric acid at a temperature in the range of 150 °C to 170 °C to obtain 2,5-dichlorophenol and a residue comprising sulfuric acid, wherein the concentration of the sulfuric acid used for hydroxylating is in the range of 60 % to 75 % w/w, and wherein the residue comprising sulfuric acid is subjected to recovery of sulfuric acid; forming an alkali metal 2,5- dichlorophenolate by reacting 2,5-dichlorophenol with an alkali metal hydroxide in at least one second fluid medium, wherein the moisture content of the alkali metal 2,5- dichlorophenolate is in the range of 0.005 to 0.05 % w/w; carboxylating the alkali metal 2,5- dichlorophenolate at a temperature in the range of 60 °C to 160 °C to obtain an alkali metal salt of 3,6-dichlorosalicylic acid; methylating the alkali metal salt of 3,6-dichloro salicylic acid, in at least one third fluid medium, with a methylating agent selected from the group consisting of methyl chloride (CH_3CI) and dimethyl sulfate [$(CH_3)_2SO_4$] at a temperature in the range of 60 °C to 160 °C to obtain methyl 3,6-dichloro-2-methoxybenzoate (dicamba ester); and hydrolysing the dicamba ester at a temperature in the range of 50 °C to 130 °C to obtain dicamba. The dicamba obtained by the process of the present disclosure has purity in the range of 98 to 99.5% [11].

Ferst stage, diazotization of 2,5-dichloroaniline with nitrosylsulfuric acid.

Initially, 2,5-dichloroaniline is diazotized with nitrosylsulfuric acid in at least one first fluid medium at a temperature in the range of -15 to 50 °C to obtain 2,5- dichlorophenyldiazonium salt (Fig. 3.1).

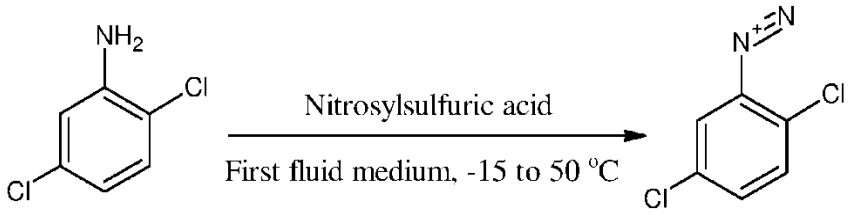


Fig. 3.1 Reaction formation of 2,5- dichlorophenyldiazonium cation

The 2,5-dichlorophenyldiazonium salt is hydroxylated by contacting with sulfuric acid at a temperature in the range of 150 °C to 170 °C to obtain 2,5-dichlorophenol and a residue comprising sulfuric acid. Concentration of the sulfuric acid used for hydroxylating is in the range of 60 % to 75 % w/w. Sulfuric acid is recovered from the residue comprising sulfuric acid. Sulfuric acid (70% w/w).

Give of Water, 150-170 °C, 2,5-Dichlorophenyldiazonium salt formation of 2, 5-Dichlorophenol. Then it is forming alkali metal 2,5-dichlorophenolate. An alkali metal 2,5-dichlorophenolate is formed by reacting 2,5-dichlorophenol obtained in next step, with an alkali metal hydroxide in at least one second fluid medium [12]. Moisture content of the alkali metal 2,5-dichlorophenolate so obtained is in the range of 0.005 to 0.05 % w/w. Give of alkali metal and 2,5 -Dichlorophenol 2,5 is forming of dichlorophenolate (Fig. 3.2).

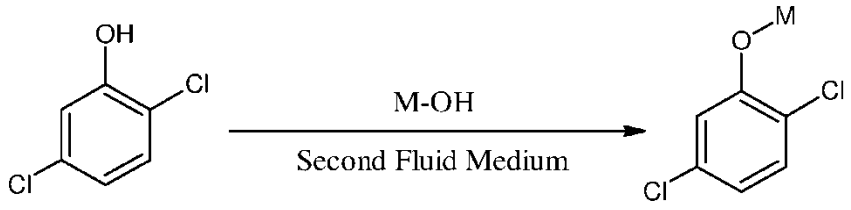


Fig. 3.2 Reaction formation of salt dichlorophenolate metal.

3.2. Synthesis of dicamba from 2,5-dichlophenol

Next carboxylation of the alkali metal 2,5-dichlorophenolate with carbon dioxide (CO_2). The alkali metal 2,5-dichlorophenolate obtained in step-3, is carboxylated with carbon dioxide (CO_2) at a temperature in the range of 60 to 160 °C, to obtain alkali metal salt of 3,6-dichlorosalicylic acid (Fig. 3.3).

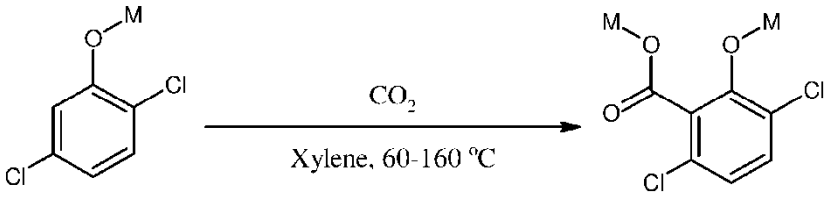


Fig. 3.3. Reaction carboxylation of Alkali metal salt 2,5-dichlorophenolate salicylic acid.

The next stage is methylation of the alkali metal salt of 3,6-dichlorosalicylic acid with a methylating agent [13]. The alkali metal salt of 3,6-dichlorosalicylic acid obtained in last step, in at least one third fluid medium, is methylated with a methylating agent selected from the group consisting of methyl chloride (CH_3CI), and dimethyl sulfate [$(CH_3)_2SO_4$] at a temperature in the range of 60 to 160 °C to obtain methyl 3,6-dichloro-2-methoxybenzoate (dicamba ester) (Fig.3.4).

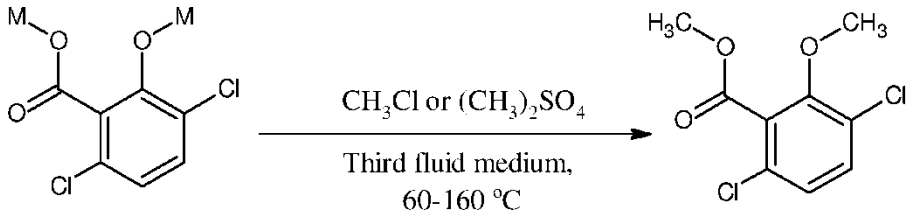


Fig. 3.4. Reaction transformation of alkali metal 3,6-dichlorosalicylic acid in 3,6-dichloro-2-methoxybenzoate.

And the last stage the dicamba ester is hydrolysed at a temperature in the range of 50 to 130 °C to obtain dicamba (Fig. 3.5).

Fig. 3.5. Reaction transformation of methyl 3,6-Dichloro-2-methoxybenzoic in 3,6-dichloro-2-methoxybenzoate acid.

Thus, a fairly pure product is obtained with a technical content of 95-98% 3,6-dichloro-2-methoxybenzoic acid, which is used both directly in this form and in the form of various derivatives for the production of various technical products and formulations.

3.3. Conclusions to the chapter

The synthesis of 3,6-dichloro-2-methoxybenzoic acid is carried out from 2,5-dichloroaniline by interaction with nitrosylsulfuric acid and the formation of 2,5-dichlophenol. Then they act with alkali to form salt. Then alkali metal-3,6-dichlorosalicylic acid is carboxylated and methylated. The final stage of the ether gyrolysis by phenolic hydroxyl.

CHAPTER 4
SAMPLE PREPARATION AND DICAMBA EXTRACTION

4.1. Sample preparation of dicamba

Pesticides such dicamba are particularly challenging and will degrade and evaporate during sample processing, extraction, and determination by GC or HPLC analysis. With care, good results can be obtained by direct GC or HPLC analysis of acetonitrile extracts, but a solvent exchange to a more HPLC -compatible solvent will permit injection of larger volumes. A solvent exchange can also reduce the concentration of coextractives, which may in turn help to reduce analyte degradation, reduce system contamination, and increase column longevity. Steiniger reported that solvent exchange from acetonitrile to hexane:acetone (9:1) removed high concentrations of polyphenols from green tea.The extraction efficiency of rapid methods for certain incurred dicamba from low moisture content samples has also been questioned. This is partly due to the fact that there is no homogenization of the sample in the solvent. In the case of low moisture commodities such as cereals, rice, tea, and spices, water should be added to the sample and the re-hydrated sample left to stand for 10–20 minutes prior to addition of acetonitrile [14]. The timing is critical especially in the case of cereals; if the time is too short the sample may not be sufficiently hydrated while if it is too long the added water can activate carboxylesterase enzymes that degrade certain.

4.2. Liquid-liquid e[traction and distribution constant

The extraction process consists in separating the investigated component from the matrix from an aqueous solution into in organic phase, according to the scheme shown in

Fig.4.1, then using a separating funnel (Fig. 4.2) separate the organic phase with the test component from the aqueous phase.

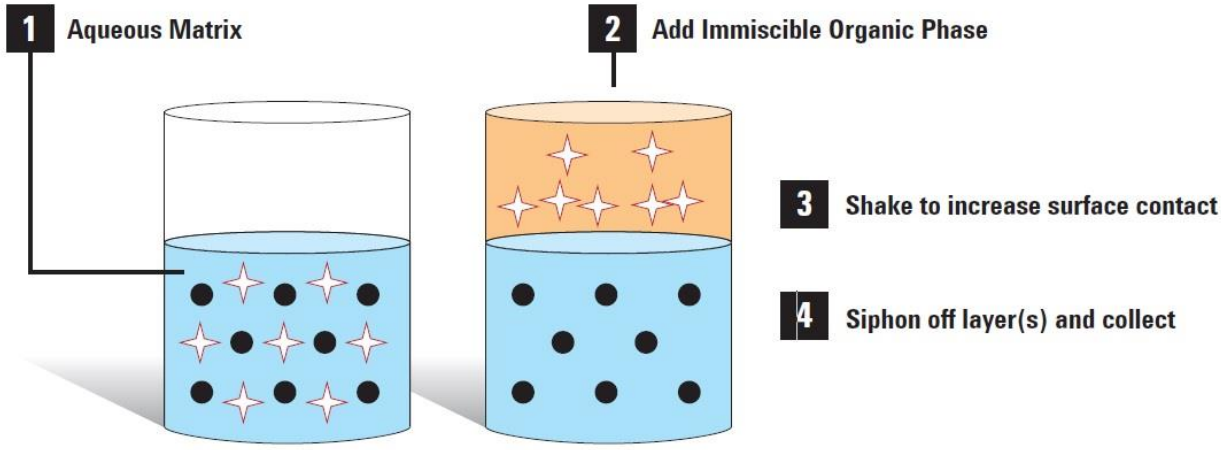


Fig. 4.1. Scheme liquid-liquid extraction.

The distribution constant is calculated using the formula [15]:

- $K_d = C_o/C_{aq}$

- where K_d is the distribution constant, C_o is the concentration (activity) of an analyte in the organic phase, and C_{aq} is the concentration (activity) of the analyte in the aqueous phase.

The distribution coefficient depends on the temperature at which the extraction is carried out, as well as on the presence of various impurities. In addition, factors such as the pH of the aqueous phase and the value of the redox potential can cause a change in the value of cr by several orders of magnitude, which gives the extraction process sufficient flexibility in the practical implementation and control of the process. The better the extractable compound is dissolved in the extractant, the greater the difference in boiling points between them, the

more chemically stable the extractable compound is to the extractant and the less it dissolves the extractant in itself, the more effective this method is.

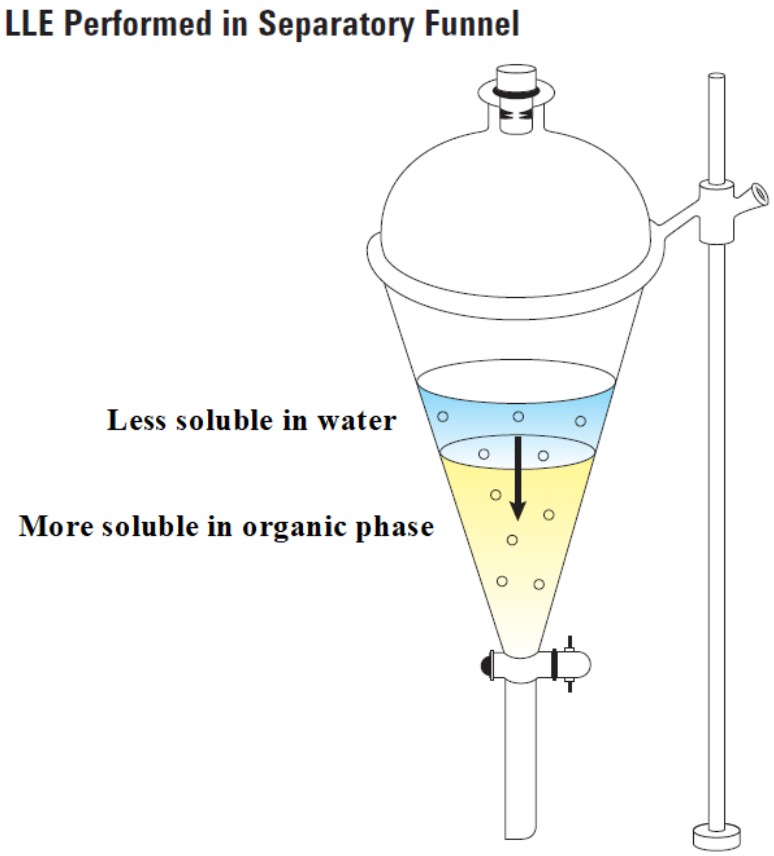


Fig. 4.2. Separation of the mixture in separating funnel.

Reduced-Scale Acetone-Based extraction the so called ‘Dutch mini Luke’ method 25, which uses a combination of acetone/petroleum ether-dichloromethane (v/v 1/1/1), has been successfully validated for a wide range of LC and GC amenable dicamba. It is preferred by some laboratories because liquid/liquid partitioning provides relatively ‘clean’ extracts without the need for additional cleanup. The lower concentration of co-extractives compared to acetonitrile and ethyl acetate results in less contamination of the instrument systems. Wider adoption of the method has possibly been hindered by the need for slightly higher volumes of solvent including dichloromethane.

For complex samples such as liver, water, and citrate, buffer salts are added followed by ethyl acetate/cyclohexane (1+1 v/v), sheme Fig. 4.3. An aliquot of the supernatant is cleaned up by GPC and then sequential dSPE with zirconium oxide and silica gel before solvent exchange into a more suitable solvent such as toluene prior to GC or HPLC analysis [16].

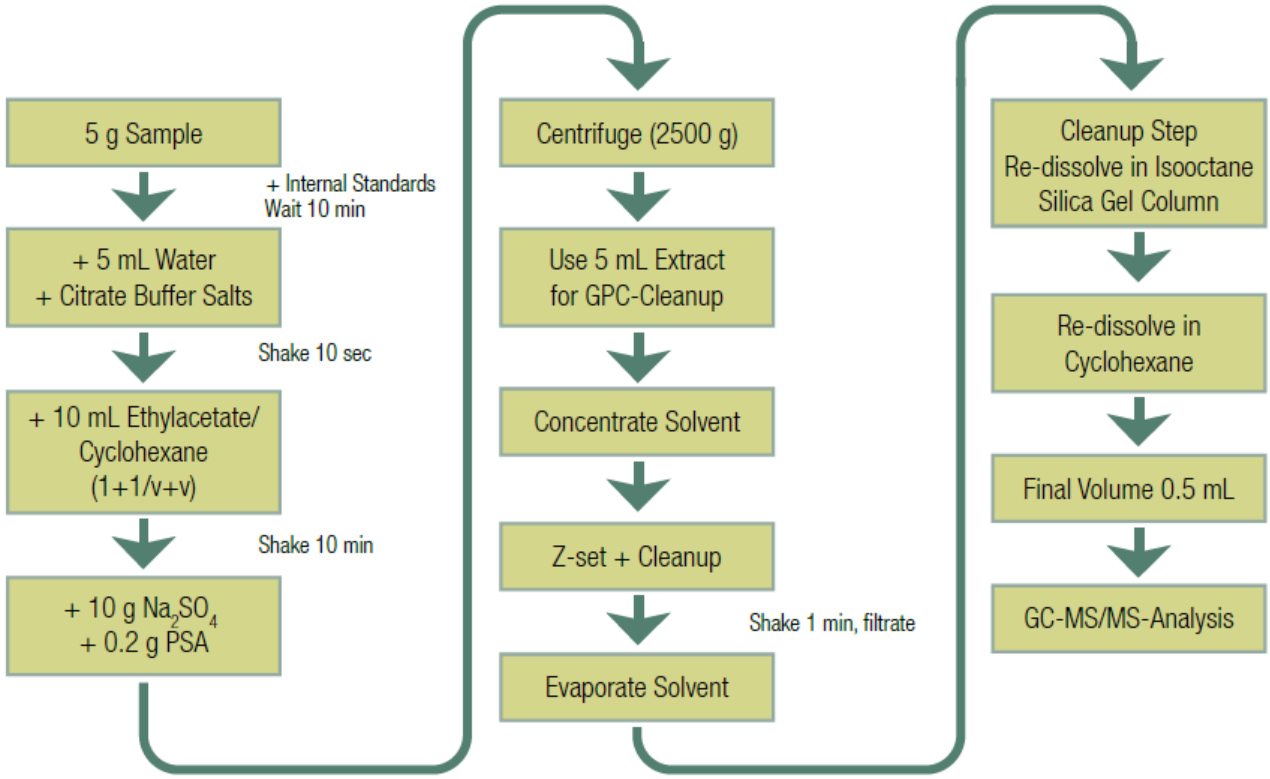


Fig. 4.3. Schemetic for the extraction of pesticides from products of plant and animal origin

The method may seem complex, but it is much more efficient than older methods using greater volumes of solvent. An excellent method to check the effectiveness of different

cleanup steps during method development for the analysis of pesticides in animal products is to use.

4.3. Conclusions to the chapter

Separation and extraction should take into account the volatility of 3,6-dichloro-2-methoxybenzoic acid, which may distort the analysis results. Liquid-liquid extraction is most commonly used to recover dicamba from various matrices. When choosing an extractant, the distribution constant must be taken into account.

CHAPTER 5
METHODS DETERMINATION OF DICAMBA

5.1. Quantitative spectrometric determination

Spectroscopic methods of analysis are important for qualitative and quantitative analysis. Spectroscopic methods are based on the ability of substances to absorb or emit electromagnetic radiation. The most often used methods are the variant when not the entire spectrum of radiation is absorbed, but its specific area. For this purpose, comparators, photocolorimeters with specific light filters and spectrophotometers are used, which can be adjusted to absorb a specific wavelength of electromagnetic radiation. In the visible area, you can measure the intensity of colored solutions. In other areas of electromagnetic radiation, measurements can be made on uncolored compounds. This is most often used for measurements in the ultraviolet region. This method is most commonly used for solutions and liquids, but it is also used for solids and gases. Each substance has its own area of absorption of electromagnetic waves. Although in many cases, if the substance is uncolored, it can be converted into a colored compound using some chemical reactions, which can already be photometric. Such a process can be carried out using complexation reactions, derivatization, and other types [17]. To carry out measurements, it is necessary to prepare a series of solutions with different concentrations of the measured component. To build a calibration curve, solutions are prepared by adding all components in the same amount to a volumetric flask, except for the substance to be measured. The substance being measured should be in some stable concentration. Photometry is carried out with respect to solutions. Comparisons. Which also contains all auxiliary components except the measured substance. Distilled water or some other pure solvent is often used instead of a reference solution. Spectrophotometric methods are based not only on cases where the intensity of the solution increases with an increase in the concentration of the test substance, but also vice versa. That

is, when the addition of a test substance destroys the colored complex and the color intensity decreases.

The absorption and emission of light is associated with electronic transitions in the atom. in this case, each atom has its own electronic sublevels, to which the electron can pass. Emitting and absorbing only in its own range of characteristic frequencies. In spectrophotometers, due to the diffraction grating, it is possible to select the conditions in which the device will be tuned only to some narrow wavelength. The finer the grating is, the narrower the range the device will be tuned to. There is also some signal processing. This is especially used in the infrared region. So in the infrared region they use signals with Fourier transform, Raman spectroscopy [18]. Spectrophotometry measures transmission. That is, the amount of light intensity that enters the solution to the intensity that is obtained at the exit from the solutionIn an array spectrophotometer, the sequence is as follows. The light source is shone into the sample and focused into a slit The transmitted light is refracted into a rainbow with the ref lection grating. The resulting light strikes the photodetector device which compares the intensity of the beam. Electronic circuits convert the relative currents into linear transmission percentages and/or absorbance/concentration values [19].

Many older spectrophotometers must be calibrated by a procedure known as "zeroing", to balance the null current output of the two beams at the detector. The transmission of a reference substance is set as a baseline (datum) value, so the transmission of all other substances are recorded relative to the initial "zeroed" substance. The spectrophotometer then converts the transmission ratio into 'absorbency', the concentration of specific components of the test sample relative to the initial substance. The schedule of spectrophotometric determination of dicamba is shown in Fig. 5.1. From the c scheme it can be seen that the beam from the light source passes through the Collimator, then through the Monochromator, then the Wavelength selector (Slit), then through the dacamba Sample solution in Cuvette then goes to the detector and the signal is fed to the Digital display or meter. Also, in addition to the display, the signal can go to a computer, with the help of which the signal can be processed and saved for further viewing or processing.

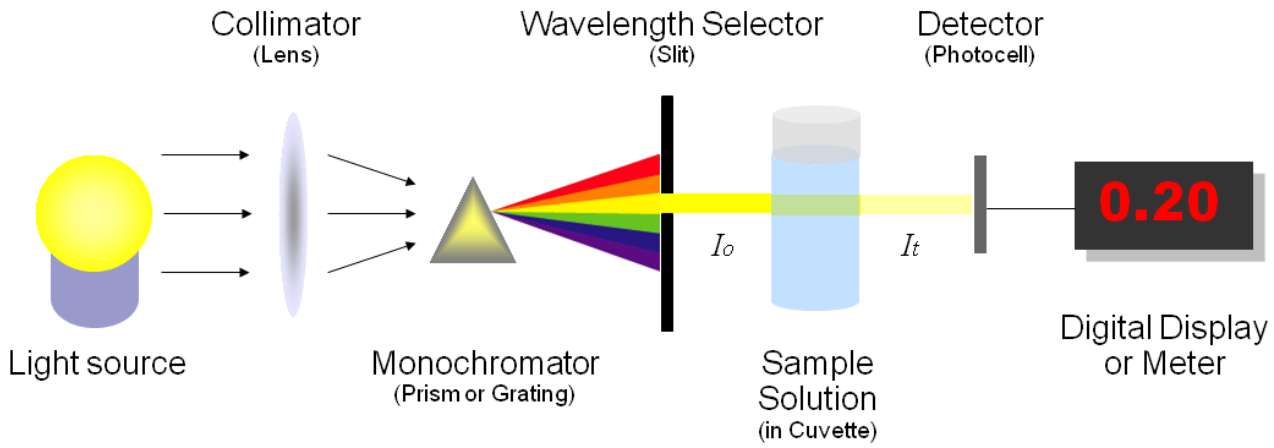


Fig. 5.1. Scheme of spectrophotometric determination of 3,6-dichloro-2-methoxybenzoic acid

Transmittance (T) is the fraction of light that passes through the sample. This can be calculated using the equation:

$$\text{Transmittance}(T)=I_t/I_o$$

where I_t is the light intensity after the beam of light passes through the cuvette and I_o is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

$$\text{Absorbance } (A)=-\log(T)=-\log(I_t/I_o)$$

Where absorbance stands for the amount of photons that is absorbed. With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample dicamba by using Beer-Lambert Law. Figure 5.2 illustrates transmittance of light through a sample dicamba. The length is used for Beer-Lambert Law described below.

If the wavelength does not change during the determination of the sample, and also, provided that the cuvette length is constant, a linear dependence of absorbance from concentration is

obtained, and thus we can determine the concentration according to the calibration curve or calculation formula.

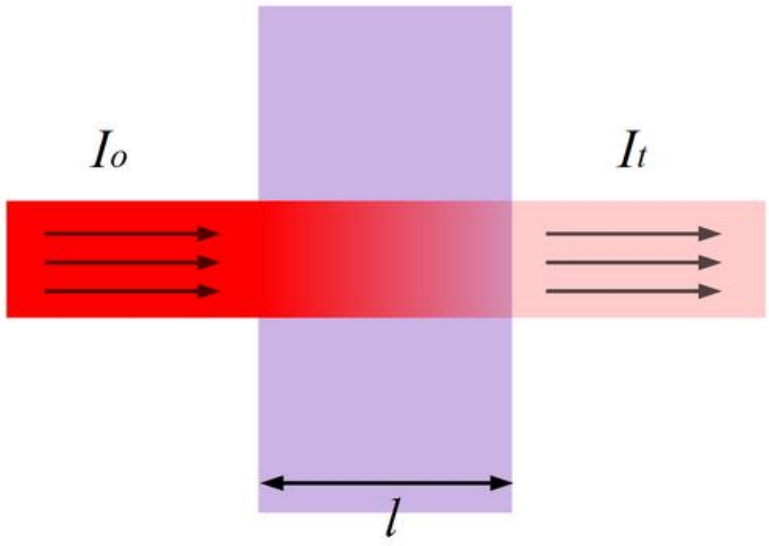


Fig. 5.2. Transmission process Scheme of dicamba determination

Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, Beer's Law can only be applied when there is a linear relationship. Beer's Law is written as:

$$\mathbf{A} = \varepsilon \bullet l \bullet c,$$

where,

A is the measure of absorbance (no units),

ε is the molar extinction coefficient or molar absorptivity (or absorption coefficient),

l is the path length, and

c is the concentration.

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units for **ε** must cancel out the units of length and concentration [20]. As a result, **ε** has the units: $L \cdot mol^{-1} \cdot cm^{-1}$. The path length is measured

in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, l is always assumed to equal 1 cm. Since absorption, ϵ, and path length are known, we can calculate the concentration c of the sample.

5.2. Spectrophotometric determination of dicamba

Both the molecular and anionic forms of dicamba have absorption maxima at wavelengths shorter than 300 nm (Fig. 5.3). In this case, the absorption maximum of the molecular form is 275 nm, and the maximum of the ionic form is slightly shifted to the long-wavelength region and amounts to 283 nm. In this case, the extinction coefficient of the molecular form of dicamba is 500 L mol^{-1} cm^{-1}. The extinction coefficient of the anionic form of dicamba is 540 L mol^{-1} cm^{-1}.

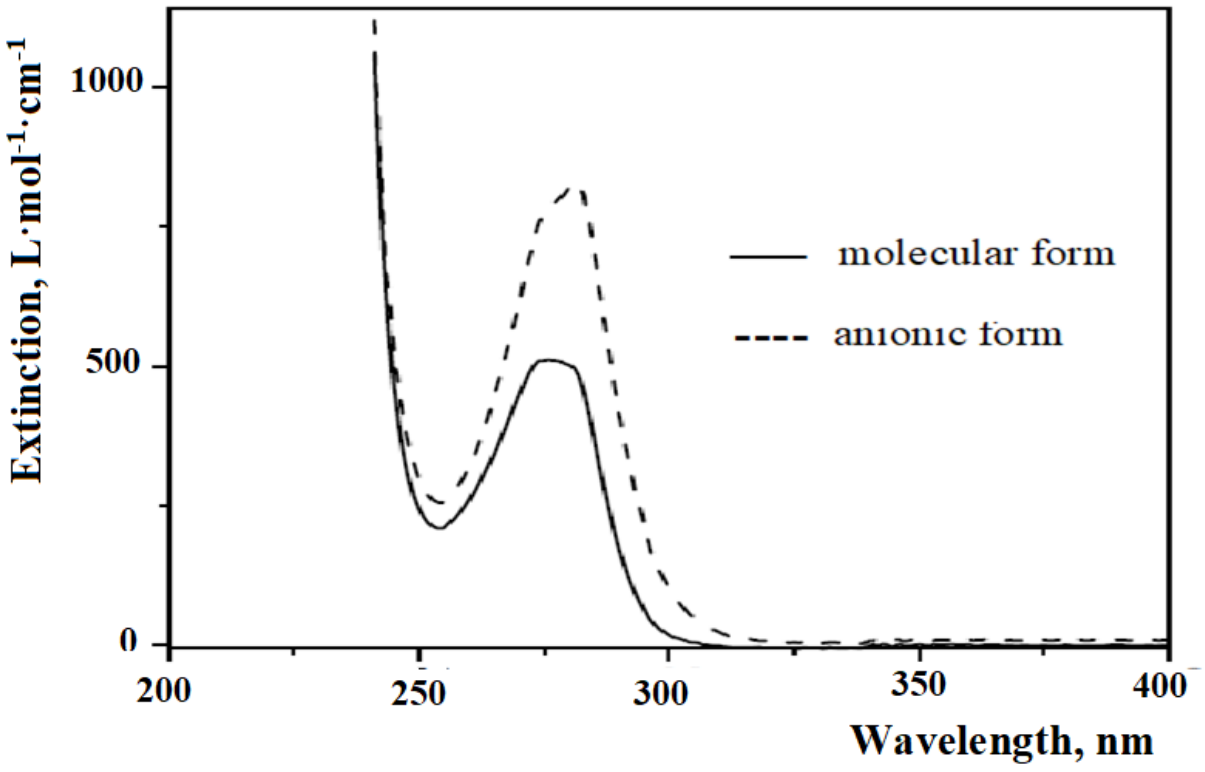


Fig. 5. 3. Molecular absorption spectrum of dicamba

Thus, the sensitivity of the determination of dicamba in the anionic form by spectrophotometric methods is slightly higher than in the anionic form. Fig. 5.2 shows that the absorption intensity increases sharply with decreasing wavelength, however, the region of hard ultraviolet radiation is inconvenient for quantitative spectrophotometric determinations [21]. Conversely, upon going to longer wavelengths, the absorption intensity sharply decreases, and, accordingly, the extinction coefficient also approaches zero. That is, based on the above, the highest accuracy in determining dicamba by the spectrophotometric method within the wavelength range of 275-290 nm.

5.3. Determination of dicamba by Raman spectroscopy methods

To obtain experimental results, a Raman spectrometer was used. The spectrum was collected using the second harmonic (532) output from a YAG:Nd laser. The dicamba sample studied was a white powder. The experimental spectrum is shown in Figure 4.1.

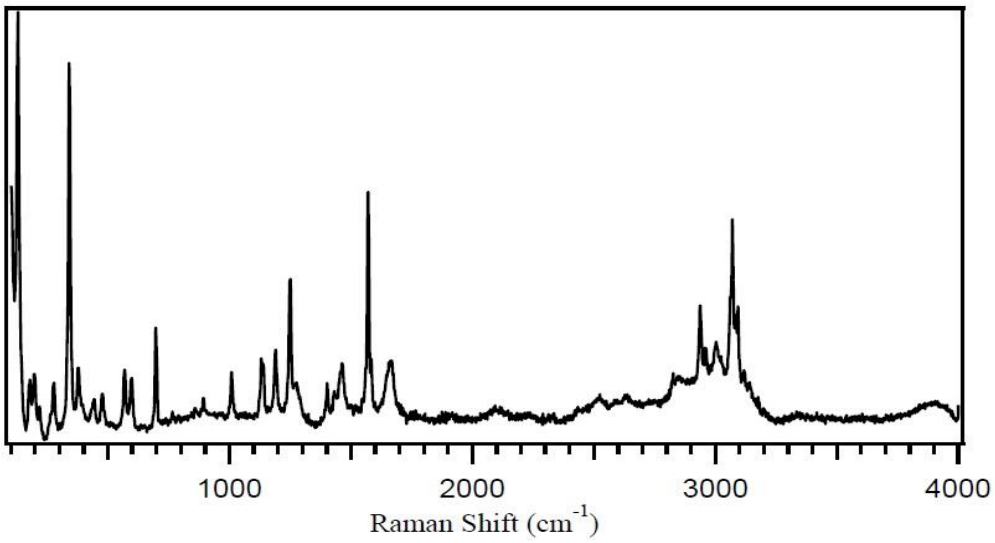


Fig. 5.2. Spectrum of dicamba recorded by method Raman spectroscopy

The figure above shows an experimental Raman spectrum of Dicamba. In this figure, the vibrational modes of interests are the peak at 1800 cm-1 and the 3500 to 4000 cm-1

range. The peak at 1800 cm-1 corresponds to the stretching of the carbonyl on the carboxylic acid functional group. The the 3500 to 4000 cm-1 range corresponds stretching of the hydroxyl functional group. In simulated envrionments seen later, sharp peaks corresponding to the hydroxyle stretch will be in the place of the broad, flatten portion of the spectrum seen above. This is most likely due to the fact that all simulated instances contain at most two Dicamba molecules interacting with one another whereas the crystalline powder studied contains far more interactions among the Dicamba molecules within the crystalline structure [22]. The reason in which multiple interactions result in a broaden portion of a spectrum is that each interaction adds a new peak to the area of interest. As more peaks are added to this region, overlap begins to occur. This overlap eventually results in the flattening of the individual sharp peaks. As more peaks are added, the region morphs from multiple sharps peaks close together to the flatten, broad region seen in the 3500 to 4000 cm-1 range on Figure 4.1. These regions of the spectrum are of great interest because water molecules and other Dicamba molecules interact with these functional groups of Dicamba.

5.4. Determination of dicamba by chromatographic method

All runs were acquired and processed using the Sciex Analyst™ software. Chromatographic separations were carried out on a Halo C8 column (50 mm x 2.1 mm x2.7 μL) at 50 °C. . The following using as mobile phase 0.2% formic acid in in methanol. The flow rate of pump C was constantly set at 0.1 mL /min. The autosampler temperature was set at 4 °C and the injection volume were 25 μL for all samples. The mass spectrometer was operated in positive ionization mode. The ionization source parameters were set the same in both periods: source temperature, 500 °C; voltage, 4500 V; curtain gas, 10 units; collision gas, 12 units; nebulizer gas 1, 70 units; nebulizer gas 2, 70 units; scan time, 150 milliseconds[23].

The quantitative and confirmative ion transitions. Sample injection and chromatography were carried out according to the scheme shown in Fig.5.3.

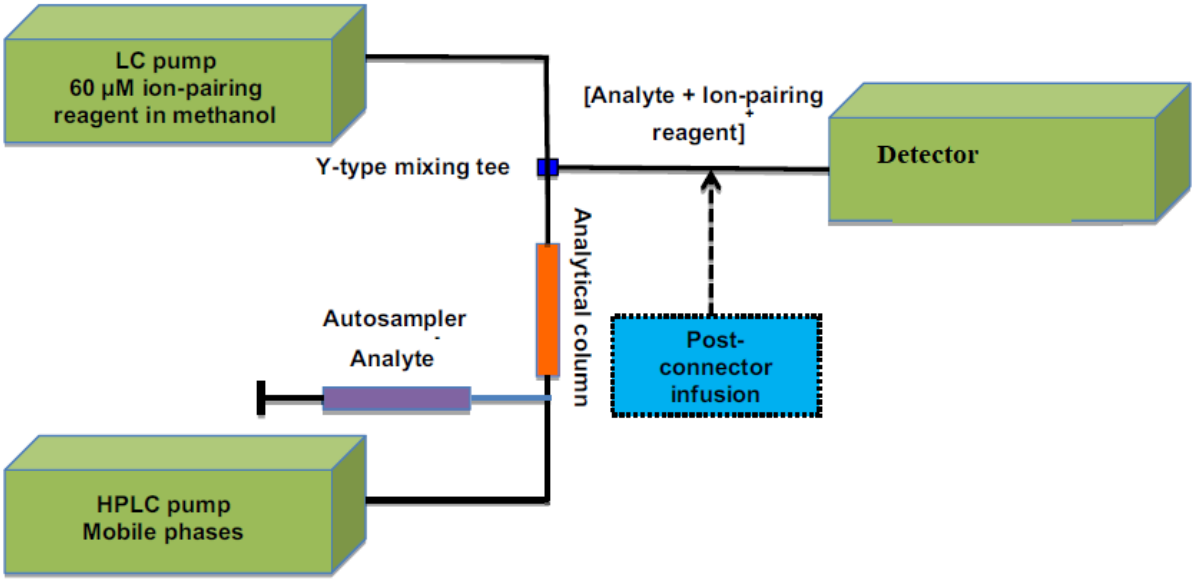


Fig. 5.3. Instrumental setup of HPLC method. The dotted lines represent the position where the standard solution was infused into the system in the matrix effects evaluation experiments

The obtained samples were chromatographed. The liquid chromatograph was injected with two samples of volume were 25 µL. The second sample was introduced 1 houar after the first one. chromatograms were obtained. almost completely identical, retention time was 8.2 min (Fig. 5.4.) in both samples, which indicates high reproducibility and correctness of chromatographic results. As we can see from the figure, both analysis results are almost completely identical not only in retention time, but also in signal intensity, as well as in peak width.

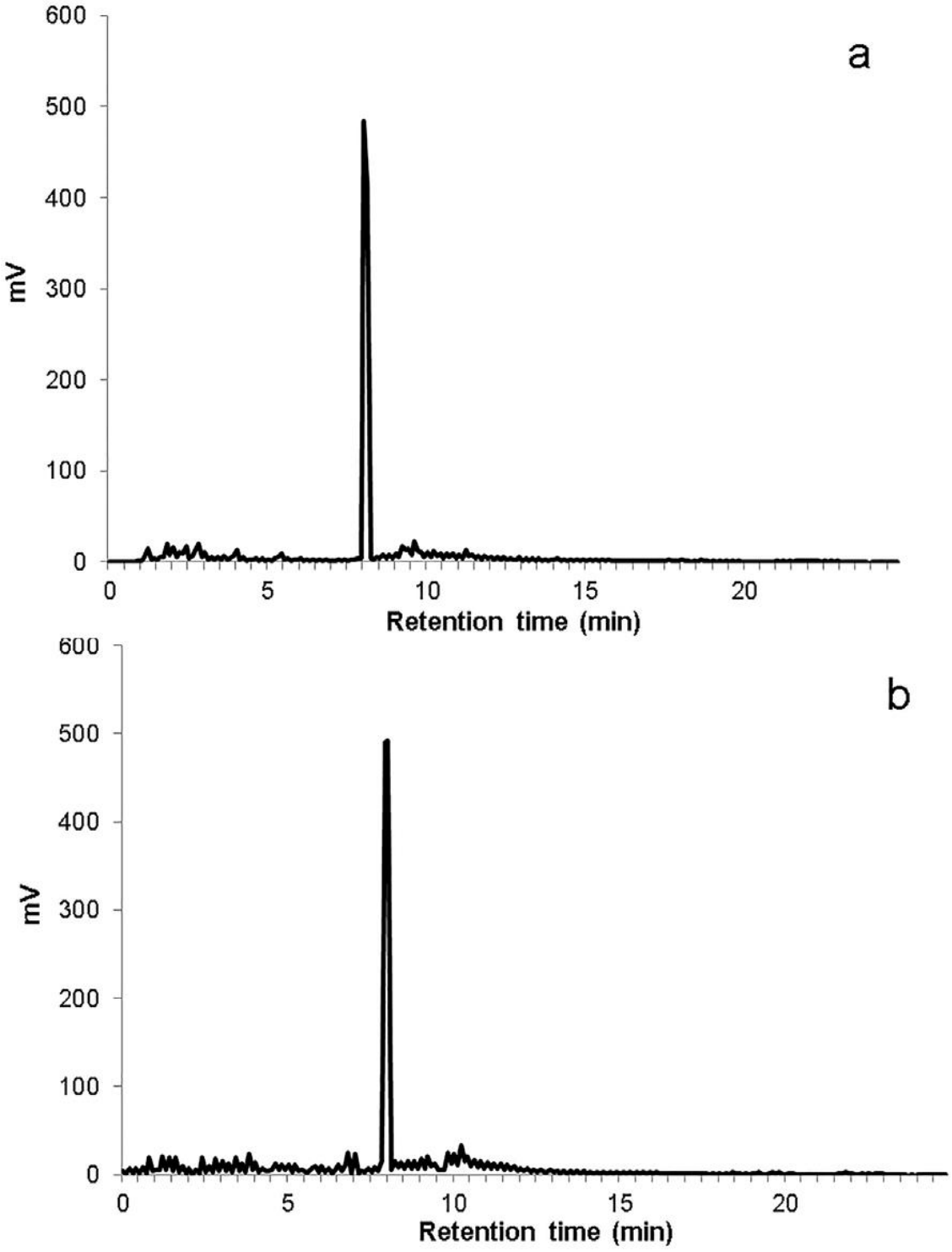


Fig. 5.4. HPLC chromatogram of dicamba for (*a*) first injection, (*b*) second injection after 1 houar. Column Halo C8 (50 mm x 2.1 mm x 2.7 μm). a flow rate of 0,1 mL/min. The dicamba retention time is 8.2 min

5.5. Determination dicamba in waters by HPLC

Preconcentration using SDB-XC EmporeTM disks Natural water samples were extracted unfiltered after acidification to pH 2.7. The pH was lower than the preconcentration method using Oasis_ HLB cartridges (pH 3) in order to aid in the recoveries of dicamba Dicamba is an extremely polar compound and the non-polar disk (SDB-XC) may not be suitable for preconcentration of this compound at pH 2.7. The other advantage of the preconcentration method using EmporeTM disks was the reduction in extraction time. The EmporeTM Filter Aid 400 allowed the natural water samples to be directly loaded onto the disk without a filtration step by acting as a depth filter to inhibit the migration of suspended solids to the surface of the disk Extraction time was dependent on sample flow, which was adjusted by the manifold vacuum pressure and could be controlled to achieve flow rates of over 100 ml $min^{-}1$ for site 1 water. However, breakthroughs of polar compounds from the Empore TM disks occurred if the samples were loaded at a high flow rate or a large water volume (several litres) was applied. This problem was overcome by gently disturbing the glass beads on the surface with a glass rod.

Chromatographic conditions. HPLC separations were performed using a system with a Waters 600 Controller equipped with a Waters 717 plus autosampler and a Waters 2487 Dual k Absorbance Detector (Waters Corporation, Milford, MA, USA). Separations were performed using an Agilent Zorbax StableBond C18 analytical column, 150 mm 4.6 mm ID, 5 μm particles (80A ˚ pore size). The mobile phase consisted of a solvent A [acetonitrile–water (80:20, v/v)] and solvent B (water) both containing approximately 3 mM HCOOH and 3 mM NH_4COOH buffer (pH 3.7)] linear gradient, from 25% to 75% of solvent A in 40 min. Flow rate was 1 ml min_1 and the volume injected was 100 μL. The analytical column was thermostatted at 25 ºC. Analytical performance and limits of detections Calibration curves were constructed from the analysis of solutions containing the range of 0.01–3.0 mg ml^{-1} of each analyte. Each standard solution was injected twice. Calibrations curves were constructed every 12 h. The retention times and peak areas of the target dicamba from these

calibration curves were repeatable within 0.1–0.3% RSD (n = 4). Retention time was 5.01 min (Fig. 1). The detection limits of the dicamba by HPLC were determined by analysing standard solutions at decreasing concentrations [24].

The limits of detection (LODs) based upon a signal to noise ratio of 3:1 were between 5 $l0^{-1}$ for dicamba. To evaluate the limits of detection after preconcentration, the same procedure was applied. Water samples collected from the Leeton sites were spiked with known concentrations of the decamp at different concentrations ranging from 0.1 to 2.4 lg l_1 and the analytes were then preconcentrated by SPE using SDB-XC EmporeTM disks.

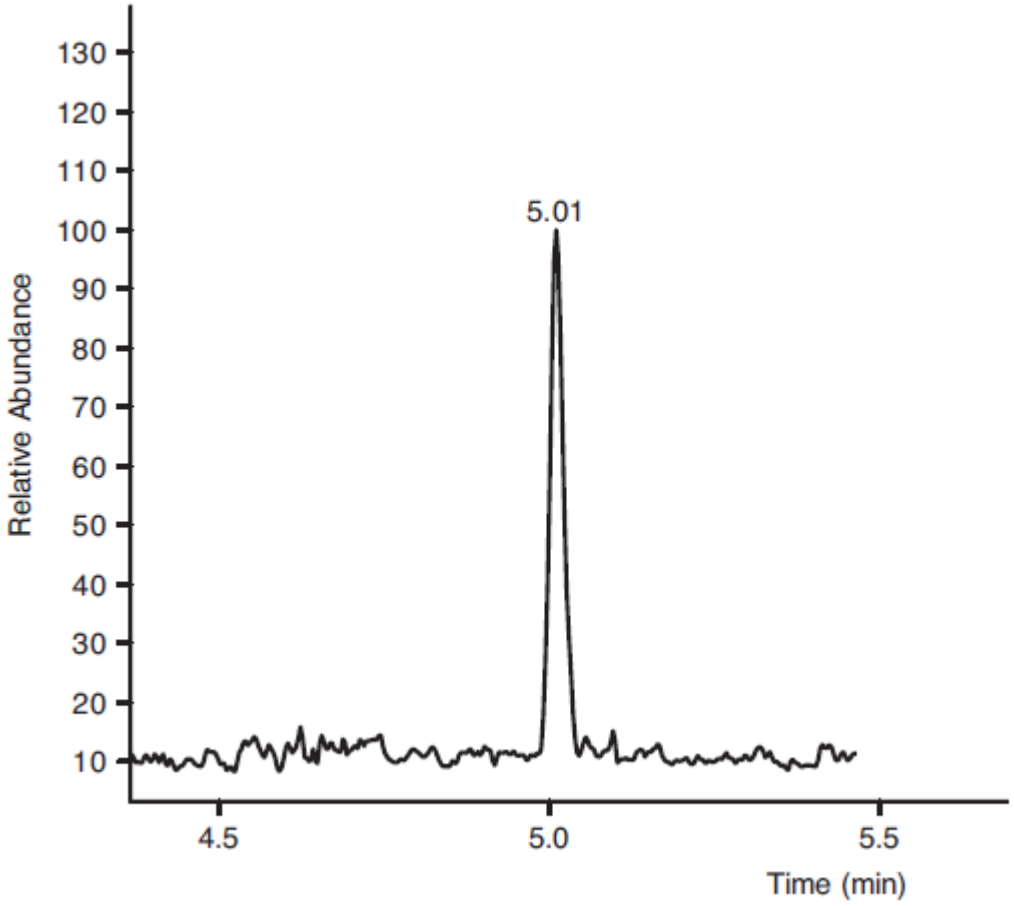


Fig. 5.5. HPLC chromatogram of dicamba in water. Column Zorbax StableBond C18 (150 mm x 4.5 mm x 5 μm). a flow rate of 1 mL/min. The dicamba retention time is 5.01 min.

5.6. Conclusions to the chapter

Spectroscopic and chromatographic methods are most often used to determine the residual amounts of 3,6-dichloro-2-methoxybenzoic acid.

Dicamba absorbs light in the ultraviolet range of 260-280 meters. The determination of its spectrophotometric method is based on this ability. The definition by Raman spectroscopy methods is also used, which is based on IR absorption in the range of characteristic frequencies of 1800 cm-1 and the 3500 to 4000 cm-1 and others.

Chromatographic methods are most widely used, in particular high-performance liquid chromatography methods. HPLC methods are characterized by good selectivity and reproducibility and high detection sensitivity.

CONCLUSIONS

1. 2-methoxy-3,5-dichlorobenzoic acid. is white crystalline substance. Well soluble in organic solvents, poorly in water. Resistant to acids and alkalis. Technical preparation (83 and 87% of d.r.) is a golden-brown crystalline powder. Contains 13-17% of impurities with a predominance of 2-methoxy-3,5-dichlorobenzoic acid.

2. Dicamba salts with alkali metals and organic bases are well soluble in water It in an aqueous medium dissociates quite well with the formation of a hydrogen cation and a dicamba anion. Dicamba has a low octanol/water partition coefficient and is resistant to oxidation and hydrolysis under most conditions.

3. Pure dicamba is low in toxicity if breathed. If inhaled, people may experience dizziness, and irritation of the nose, resulting in coughing. If pure dicamba is contaminated with human skin, it has low toxicity, but skin irritation may develop. If dicamba is swallowed, people have reported symptoms such as vomiting, loss of appetite and muscle spasms. If a large amount is swallowed, diarrhea and abdominal pain have been reported.

4. Pets may be exposed to dicamba if they come into contact with plants that have been treated with dicamba, either by eating the plants or walking through an area where dicamba was applied. Signs that a dog or a cat may have been exposed to dicamba include shortness of breath, muscle spasms and the animal may produce a lot of saliva.

5 .The herbicidal activity of dicamba is similar to 2,3,6-trichlorobenzoic acid, but slightly superior to it in potency. Dicamba classified as a herbicide with auxin-like activity. Its effect is manifested in increasing the rate of RNA synthesis and its concentration, accelerating the synthesis of lipids and protein, increasing the extensibility of membranes and cell growth in length.

6. Dicamba is characterized by mobility in plants. Penetrating through the leaves into plants, it moves quickly into the root system. Moves along the phloem and xylem,

accumulating mainly in the growing tips. From the roots of a small amount of the drug can pass into the environment.

7. When using dicamba as a herbicide, only a small part of the substance gets on the plant and has an herbicides effect. Most of the drug is washed into the soil and stays there for a long time, gradually decomposing and metabolized by microorganisms.

8. While in the soil, it has a detrimental effect on the positive microflora. Surface water and groundwater are also washed away from the soil. In addition, the amount of dicamba that gets on the plants can be absorbed by them and then get into the fruits of plants unevenly distributed inside the plant in residual amounts of 3,6-dichloro-2-methoxybenzoic acid.

9. A process for the preparation of 3,6-dichloro-2-methoxybenzoic acid (dicamba) involves diazotizing 2,5-dichloroaniline with nitrosylsulfuric acid in at least one first fluid medium at a temperature in the range of -15°C to 50 °C to obtain 2,5-dichlorophenyldiazonium salt; hydroxylating the 2,5- dichlorophenyldiazonium salt by contacting the 2,5-dichlorophenyldiazonium salt. Then it is hydroxylated, carboxylated, methylated. The final stage of hydrolysis of dicamba methyl ester.

10. The extraction process of dicamba consists in separating the investigated component from the matrix from an aqueous solution into in organic phase, then using a separating funnel separate the organic phase with the test component from the aqueous phase. When choosing an extractant, the distribution constant must be taken into account.

11. Determination of dicamba spectrophotometrically based on its ability to absorb Electromagnetic vibration in the ultraviolet region in the wavelength range 260-280 nm, Raman spectroscopy, a peak is recorded at 1800 cm-1 and other characteristic frequencies of dicamba.

12. For the determination of dicamba by high performance liquid chromatography, a type of reverse phase chromatography is used. A mixture of acetonitrile-water or methanol-water is used as the mobile phase.HPLC methods are characterized by good selectivity and reproducibility and high detection sensitivity.

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