172568

DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN TISSUES OF BUFFALOES AND DUCKS

POULSON JOSEPH

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary & Animal Sciences
Kerala Agricultural University
Thrissur

2006

Department of Livestock Products Technology
COLLEGE OF VETERINARY & ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN TISSUES OF BUFFALOES AND DUCKS** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

POULSON JOSEPH

Mannuthy,
24.10. 2006

CERTIFICATE

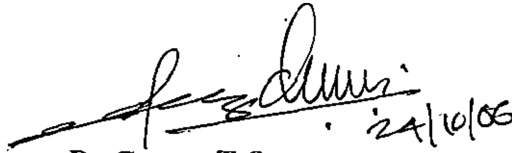
Certified that this thesis, entitled **DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN TISSUES OF BUFFALOES AND DUCKS** is a record of research work done independently by **Dr.Poulson Joseph**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Mannuthy
24-10. 06

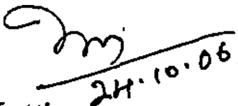
Dr.George T.Oommen
(Chairman, Advisory Committee)
Associate Professor
Department of Livestock Products Technology
College of Veterinary & Animal Sciences
Mannuthy, Thrissur

CERTIFICATE

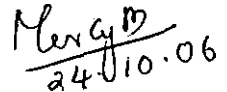
We, the undersigned members of the Advisory Committee of Dr.Poulson Joseph, a candidate for the degree of Master of Veterinary Science in Livestock Products Technology, agree that the thesis entitled **DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN TISSUES OF BUFFALOES AND DUCKS** may be submitted by Dr.Poulson Joseph, in partial fulfilment for the degree.



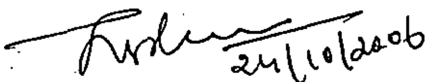
Dr.George T.Oommen
(Chairman, Advisory Committee)
Associate Professor
Department of Livestock Products Technology
College of Veterinary & Animal Sciences
Mannuthy



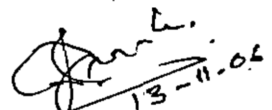
Dr.P.Kuttinarayanan
Associate Professor & Head
Department of Livestock
Products Technology
(Member)



Dr.A.D.Mercy
Associate Professor & Head
Department of Animal Nutrition
(Member)



Dr.Joseph Mathew
Assistant Professor (Senior scale)
Department of Livestock
Production Management
(Member)



External Examiner

ACKNOWLEDGEMENT

It is beyond the power of my expression for acknowledging my Major Advisor and Chairman of the Advisory Committee **Dr. George T. Oommen,** Associate Professor, Department of Livestock Products Technology. His valuable guidance, creative suggestions, constructive criticism and constant encouragement during the course was praiseworthy and valuable. It was indeed a pleasure for me to work under his superb guidance and I am sure that the qualities which I gained through such training will last, life long.

I am fortunate to have **Dr.P.Kuttinarayanan**, Associate Professor& Head, Department of Livestock Products Technology as a Member of the Advisory Committee. He provided a persistent, gentle support during my entire course of work. He has remained extremely approachable and friendly. I thank him for his words of wisdom and also for rendering the facilities for the research work as Head of the Department.

My heart felt gratitude to **Dr.A.D.Mercy,** Associate Professor & Head, Department of Animal Nutrition as Member of the Advisory Committee for her valuable suggestions, timely corrections and continued support provided during the research work.

I am sincerely thankful to **Dr Joseph Mathew**, Department of Livestock Production Management as a Member of the Advisory Committee for his whole-hearted co-operation during the period of research work.

To the greatest degree, I am grateful to **Dr. Sanis Juliet**, Assistant Professor, Department of Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Pookot for her personal guidance in HPLC analysis, generous encouragement and inspiration in the pursuit of this work inspite of all her official responsibilities at Pookot College.

I am grateful to the **Dean,** College of Veterinary & Animal Sciences, Mannuthy, and **Kerala Agricultural University** for providing the financial assistance and facilities for the conduct of this research work.

My heart felt gratitude to **Dr.Ranjith Ramanathan** for his affectionate and sincere support throughout my work. I owe my sincere thanks to my classmates **Drs. Kavitha Rajagopal, P.Jenifer, Vivek A.K** and **Rana Raj.V.R** for their valuable timely help and moral support.

The invaluable help rendered by my beloved juniors **Drs.Dinkar Salke** and **Naseera A.P.** is duly acknowledged. Also sincere thanks to **Drs. Kishore K, Madhusudhanan, M.K, Sany Thomas, Aneesh, A** and **Mrs. Sreeja** for their support during the research work.

I sincerely acknowledge all the helps rendered during the course of my research work by **C. J. Joseph, Ranjithkumar, T,** Processing Associates, trainees, Para-technical and other Office staff in the Department.

The help rendered by **Kishore** and **Abhilash** during sample collection is thankfully acknowledged. I express my deep sense of gratefulness to **Sinu, Biju** and **Renolson.** I acknowledge my deep gratitude to Anil, Sivadas, Santhosh, Saif, Vinod, Jacob, Arun, Praveen, Rajagopal, Ajmal, Binoj and Abdulla for their whole-hearted support.

I thank my P.G. batchmates and all PG hostel inmates for their love and support given to me. Thanks to Drs. Hareesh, Prejith, Shekar and Shambunath for their varied help, support and inspiration.

I am deeply indebted to my beloved parents and siblings for their affection support, sacrifice and encouragement.

Above all, I bow before the almighty for the interminable blessings showered upon me throughout my life.

POULSON JOSEPH

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
	2.1 PESTICIDE USE PATTERN	4
	2.2 CARBOFURAN	4
	2.2.1 General Description	5
	2.2.2 Environmental Fate	5
	2.2.3 Metabolism in Animals	5
	2.3 TOXICITY OF CARBOFURAN	6
	2.3.1 Toxicity in Fish and Invertebrates	6
	2.3.2 Toxicity in Mammals	7
	2.3.3 Avian and Wild Life Toxicity	8
	2.4 RESIDUE LEVELS OF CARBOFURAN	9
	2.4.1 Environment	9
	2.4.2 Residues in Crops and Fodder	9
	2.4.3 Residues in Animals	10
	2.5ANALYSIS OF RESIDUES IN ANIMAL TISSUES	11
	2.5.1 Sample Collection	11
	2.5.2 High Performance Liquid Chromatography (HPLC)	12
	2.5.2.1 Extraction and Clean up	13
	2.5.2.2 Detection	14
	2.5.2.3 Recovery Studies	14
	2.5.3 Maximum Residue Limits (MRL)	15

3	MATERIALS AND METHODS	16
	3.1SAMPLE COLLECTION AND PREPARATION	16
	3.1.1 Sampling	16
	3.1.2 Initial preparation of the samples	17
	3.2 EXTRACTION METHOD	17
	3.2.1 Extraction with acetonitrile	17
	3.2.2 Extraction with acetone, methanol, 2-propanol and hexane	18
	3.3 CLEANUP	18
	3.3.1 Meat, Kidney and Liver	18
	3.3.2 Fat	18
	3.4 HPLC ANALYSIS	19
	3.5 STANDARDISATION	20
	3.5.1 Linearity study	20
	3.5.2 Recovery Study	21
	3.6 SAMPLE SCREENING	21
	3.7 STATISTICAL ANALYSIS	21
4	RESULTS	22
	4.1 STANDARDISATION OF HPLC CONDITIONS	22
	4.2 LINEARITY STUDY	23
	4.3 RECOVERY STUDY	23
	4.4 SOLVENT SELECTION	24
	4.5 SAMPLE SCREENING	25
5	DISCUSSION	51
	5.1 HPLC OPERATIONAL CONDITIONS	51
	5.2 STANDARDISATION	52
	5.2.1 Linearity Study	52

	5.2.2 Recovery Study	53
	5.3 SAMPLE SCREENING	53
6	SUMMARY	55
	REFERENCES	58
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Peak area values of known concentrations of carbofuran in acetonitrile on estimation by HPLC	26
2	Peak area values of known concentrations of 3-hydroxy carbofuran in acetonitrile on estimation by HPLC	26
3	Recovery rate (%) of carbofuran from different matrices using different solvents.	27
4	Mean recovery (%) of carbofuran from different matrices on fortification with known concentrations	27
5	Concentration of residues of carbofuran and its metabolites in buffalo samples	28
6	Concentration of residues of carbofuran and its metabolites in duck samples	28

LIST OF FIGURES

Figure No.	Title	Page No.
1	Chromatogram of 3-hydroxy carbofuran (100 ppm) at a flow rate of 1ml/min.	29
2	Chromatogram of carbofuran (10ppm) at a flow rate of 1ml/min.	30
3	Chromatogram of carbofuran (100 ppm) at a flow rate of 1.3ml/min.	31
4	Chromatogram of carbofuran (100 ppm) and 3-hydroxy carbofuran (100 ppm) under programmed flow rate	32
5	Chromatogram of carbofuran (100 ppm) at programmed flow rate	33
6	Chromatogram of 3-OH-7-phenolcarbofuran, 3-keto-7-phenol carbofuran and carbofuran phenol	34
7	Chromatogram of 3-keto carbofuran	35
8	Chromatogram of carbofuran phenol	36
9	Linearity of peak area values against known graded concentrations of carbofuran	37
10	Linearity of peak area values against known graded concentrations of 3-hydroxy carbofuran	37
11	Recovery of carbofuran from buffalo meat after fortification and extraction with acetonitrile	38
12	Chromatogram of buffalo meat used for carbofuran fortification and recovery study	39
13	Chromatogram of buffalo liver used for carbofuran fortification and recovery study	40
14	Chromatogram of buffalo kidney used for carbofuran fortification and recovery study	41

15	Chromatogram of buffalo fat used for carbofuran fortification and recovery study	42
16	Chromatogram of buffalo meat screened for the residues of carbofuran and its metabolites	43
17	Chromatogram of buffalo liver screened for the residues of carbofuran and its metabolites	44
18	Chromatogram of buffalo kidney screened for the residues of carbofuran and its metabolites	45
19	Chromatogram of buffalo fat screened for the residues of carbofuran and its metabolites	46
20	Chromatogram of duck meat screened for the residues of carbofuran and its metabolites	47
21	Chromatogram of duck liver screened for the residues of carbofuran and its metabolites	48
22	Chromatogram of duck kidney screened for the residues of carbofuran and its metabolites	49
23	Chromatogram of duck fat screened for the residues of carbofuran and its metabolites	50

Introduction

INTRODUCTION

Residues of pesticides and its metabolites in food have been identified as major health hazard and risk factor, all over the world. With rampant use of high doses of pesticides in agriculture and increasing incidence of ailments in human population, it has become mandatory to assess the residue levels in foods in order to ward off hazards as per Food Safety Management and Hazard Analysis Critical Control Point. Meat being a food of choice internationally, the food safety parameters extend from "Farm to Fork". As such, it is imperative to identify and control the risk factors including pesticide residues in animal production and subsequent product processing.

Indian agriculture scenario is closely interwoven with pesticide and fertilizer application, which assisted in achieving green revolution. Initially organochlorine compounds were used, but due to their bioaccumulation these were banned or restricted in most of the technically advanced countries in 1960. Later introduction of synthetic insecticides such as organophosphorus compounds in 1960s, carbamate in 1970s, pyrethroids in 1980s and herbicides and fungicides in 1970-80s contributed greatly in pest and vector control and agricultural output. Even though the pesticides enhanced economic potential in terms of increased production of food and fibre and amelioration of vector borne diseases, they have resulted in serious health implication to man and environment (Ganguly *et al.*, 2001). Pesticide residues in agricultural crops and livestock products can be direct, indirect or from the environment.

Carbofuran is a broad-spectrum carbamate pesticide, which is widely used as systemic insecticide, nematicide and acaricide by soil, foliar and seed treatment application on cereals and vegetables. The common formulations marketed are in flowable and granular forms under various trade names. It is found to persist in soil, water and agriculture products.

World Health Organisation (1996b) has classified carbofuran as a highly hazardous compound. Toxicity studies in mammals, fish and invertebrates revealed that carbofuran is acutely toxic and produced chronic toxicity in laboratory animals. Its reproductive toxicity, immunotoxicity and genotoxicity reveal the necessity for residue studies.

Carbofuran can enter the food chain of animals through water, soil, feed and fodder and the residues have been detected in these sources. Though carbofuran does not bioaccumulate in animal tissues, metabolism studies proved that residues are detected in liver, kidney, fat and muscles (Khatoon and Chowdhury, 2001) and the metabolites reported are 3-hydroxy carbofuran, 3-keto carbofuran, carbofuran phenol, 3-hydroxy 7-phenol carbofuran and 3-keto phenol carbofuran (Ahdaya and Guthrie, 1982). The residues are stable for at least one year in meat and two years in frozen plant commodities and milk (FAO, 2004).

FAO Statistics Database, FAOSTAT (2006) shows that buffalo meat production is increasing and has reached 1.487 million tonnes in 2005 in India.. It is preferred to beef by the consumers and also forms a major share of meat exported from India. There is no religious restriction attached to the buffalo meat consumption. So also, duck meat production in India has increased in 2005 up to 65,000 tonnes. The managemental practices of duck and buffalo rearing can make them more vulnerable to the exposure of pesticides. Ducks can ingest the granules of pesticides while sifting sediments (Elliot *et al.*, 1996). The most common route of exposure of wild birds to pesticides is by ingestion of poisoned insects, carcasses, or grains intentionally treated with pesticides for bait (Hill and Fleming, 1982; Quick, 1982). Presently, the meat production of our state depends on animals brought from other states where extensive pesticide application is practiced.

So regular monitoring of residues of carbofuran and its metabolites in tissues of buffaloes and ducks reared under different management systems in varied geographical areas is indispensable for risk analysis. This is to assess the

incidence percentage of non compliant residues in meat and poultry which further enables to ascertain the primary sample size from a lot for residue analysis to determine the compliance with Codex Maximum Residue Limits (CAC, 1999). This is inevitable to ensure food safety, as residue in excess of Maximum Residue Limits may cause deleterious effects in consumers.

Several residue analytical methods are reported for carbamate pesticides in food, fodder, soil and water. But High Performance Liquid Chromatography (HPLC) method is found to be the most suitable method for residue screening for carbamate compounds (Aaron and Coly, 2000). Different extraction methods and cleanup procedures have been developed by research workers employing different solvents, viz., acetonitrile, n-hexane, acetone and different techniques such as solid phase extraction. If the animals are exposed to different types of pesticides during their growth and development and in animal tissue during processing, a multiresidue method of analysis is required. Only few methods are developed for extraction of carbofuran residues from animal tissues for analysis in HPLC.

Therefore, the objectives of the present study are to 1) develop a suitable simple method of extraction of residues of carbofuran and its metabolite from meat, liver, kidney and fat of buffaloes and ducks for reversed phase HPLC analysis and standardise the method and 2) application of this method in the determination of the residues of carbofuran and its metabolite in animal tissues. This would enable in monitoring and surveillance of residues of carbofuran in animal tissues, hazard analysis and determining the critical control points.

Review of Literature

REVIEW OF LITERATURE

A research work was undertaken to determine the residues of carbofuran and its metabolites in meat, fat, liver and kidney of buffaloes and ducks by High Performance Liquid Chromatography (HPLC). There is dearth of literature on HPLC analysis for the detection of residues of carbofuran and its metabolites in animal tissues. However, a thorough scanning of literature was done on the various aspects of the work including pesticide use pattern, carbofuran and its metabolism in animals and environment, toxicity, residue levels and residue analysis by reverse phase HPLC.

2.1 PESTICIDE USE PATTERN

A substantial rise occurred in the production and use of pesticides in India during the last three decades and the domestic demand in India accounts for 76 per cent of the total pesticides used in the country as against 44 per cent globally (Mathur, 1999).

Sivakumar and Jiji (2003) in a field survey for documenting farmers' practices on pest management reported that all farmers in Thiruvanathapuram region, Kerala, incorporated carbofuran to the soil at the time of sowing snake gourd during the two vegetable growing seasons of 1999/2000. The pesticide consumption in Kerala showed a declining trend in the recent years due to the dissemination of integrated pest management and organic farming practices. But the intensity of use increased to about 3.5 kg/ha (Indiradevi, 2006).

2.2 CARBOFURAN

A general information on physicochemical characteristics of carbofuran and its metabolism in environment, plants and animals is essential in deciding the extractants, cleanup method and suitable detectors for HPLC residue analysis.

2.2.1 General Description

Carbofuran is a broad spectrum, noncumulative systemic carbamate insecticide which is chemically 2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate. It is soluble in water and organic solvents like acetonitrile, acetone, and hexane at 25° C and is stable under neutral or acidic conditions but unstable in alkaline media. The common formulations marketed are in flowable and granular forms under the trade names of Bay 70143, Carbofuran, Furudan, Curaterr, FMC10242, and YaltoxiR (FAO/WHO, 1985). It is used world wide as insecticide, nematicide and acaricide for many crops like rice, potatoes, alfalfa, corn, peanuts, banana, sorghum, sugarcane, pepper and cottonwood trees and the technical product contains a minimum of 95 per cent carbofuran (FAO/WHO, 1997).

2.2.2 Environmental Fate

Carbofuran is degraded in water by hydrolysis, microbial decomposition and photolysis. The hydrolysis half-lives in water at 25° C of 690, 8.2 and 1 week have been reported at pH levels of 6.0, 7.0 and 8.0, respectively (Health and Welfare Canada, 1991).

The degradation products of carbofuran in soil include carbofuran phenol, 3-hydroxy carbofuran, and 3-keto carbofuran (IPCS, 1997). Carbofuran may leach significantly, although leaching is reduced highly in organic soils (Kannathasan *et al.*, 2001).

The half-life of carbofuran has been reported as 320 days in acidic conditions and 150 days under alkaline conditions while the photolysis half-life of carbofuran in soil was about 78 days (FAO, 2004).

2.2.3 Metabolism in Animals

Ahdaya and Guthrie (1982) reported that carbofuran administered to female mice by gavage was rapidly absorbed. They suggested that metabolism

involves hydroxylation and/or oxidation reactions that result in the formation of carbofuran phenols, hydroxy carbofuran, 3-hydroxy 7-phenol carbofuran, 3-ketofuran and 3-ketofuran-7 phenol. The metabolic pathway consists of hydroxylation, oxidation, hydrolysis and conjugation (FAO/WHO, 1997).

In a study conducted to evaluate the bioavailability and toxicological potential of wheat bound 14C carbofuran residues in rats, Khatoon and Chowdhury (2001) observed that the per cent of dietary bound 14C residues in fat (4.8 %), liver (8.0 %), kidney (1.2 %) and blood (8.0 %) were higher compared to that in other organs, viz., muscle (0.72 %), spleen (0.48 %) and heart (0.32%).

Animal metabolism studies in hen revealed the presence of residues of carbofuran in liver, fat, muscle and skin. The major metabolites detected were 3-hydroxy carbofuran, 3-hydroxy 7-phenol carbofuran and 3-keto 7-phenol carbofuran, 7-phenol and 3-keto carbofuran in liver (FAO, 2004).

2.3 TOXICITY OF CARBOFURAN

Carbofuran is a cholinesterase inhibitor with contact and stomach action and highly toxic to mammals after acute oral administration (FAO/WHO, 1985). World Health Organisation (1996b) has classified carbofuran as highly hazardous compound and toxicity studies reveal that carbofuran is acutely toxic orally to mammals, fish and invertebrates. Several incidents of wildlife deaths and poisoning have been reported from carbofuran exposure (Littrel, 1988). Carbofuran was grouped among genotoxic compounds and developmental or reproductive toxicants under the toxicologic group of pesticides (Gunier *et al.*, 2001). Chronic toxicity, reproductive toxicity and immunotoxicity are also evident in lab animals.

2.3.1 Toxicity in Fish and Invertebrates

The fresh water fish, *Labeo rohita* was exposed to sublethal dose of furudan, 0.5 ppm for 60 days to study the haematological changes and found that

erythrocytes and hemoglobin content decreased significantly (Bhatkar and Dhande, 2000).

Ram *et al.* (2001) studied the effect of chronic exposure to 4.5 ppm carbofuran in static water in the teleost, *Channa punctatus* (Bloch) and observed significant inhibition of gonadal development with associated degenerative abnormalities.

Sudipta *et al.* (2001) opined that carbofuran acts as an antiestrogenic, endocrine disrupting agent in catfish, *Heteropneustes fossilis* (Bloch) possibly targeting the pituitary-gonad axis.

Tripathi *et al.* (2002) reported that carbofuran caused shrunken mitochondria and golgi complex in earth worm *Lampito mauritii.*

2.3.2 Toxicity in Mammals

Street and Sherma (1975) showed that rabbits treated with DDT, carbofuran and methyl parathion had decreased counts of activated lymphocytes in the lymph nodes, reduced number of germinal centers in the spleen and more pronounced atrophy of the cortex of thymus.

Reduced body weight gain in dams and a slightly increased incidence of skeletal variations in pups of New Zealand White rabbits were observed when fed with carbofuran at a dose level of 2 mg/kg of body weight/day (Laveglia, 1981).

Goldenthal (1982) observed that body weight gain and plasma, erythrocyte and brain cholinesterase activities were reduced in Charles River rats in a long term oral exposure toxicity study of carbofuran while short term exposure study was assessed by Bloch (1987) by feeding carbofuran to a group of beagle dogs at different dietary concentrations.

Carbofuran suppresses T-cell-mediated immune responses in male mice by the suppression of T-cell responsiveness, the differential inhibition of cytokine production, and nitric oxide production in macrophages (Jeon *et al.*, 2001).

Baligar and Kaliwal (2003) reported that the treatment of Swiss albino mice with carbofuran at 1.3 mg/kg/day administered by gavage for 30 days caused a significant decrease in the number of estrous cycle and duration of pro-estrous, estrus and metoestrus. They also reported that prolonged carbofuran treatment significantly reduced body weight and ovary weight in Swiss albino mice.

2.3.3 Avian and Wild Life Toxicity

Hunt *et al.* (1995) detected the exposure of herons to carbofuran using brain cholinesterase reactivation techniques in six black crowned night heron carcasses collected from a potential pesticide exposure incident.

Considering the wildlife mortality incidents from 1971 to 1989, carbofuran and fensulfothion were implicated in the deaths of thousands of waterfowls and songbirds in the Fraser Delta, Canada, where intensive vegetable farming was undertaken (Wilson *et al.*, 1995).

Elliot *et al.* (1996) reported that granular carbofuran persisted long enough in the wet, low pH condition of the Fraser Delta, Canada to kill waterfowls and cause secondary poisoning of raptors several months after the application of the pesticides.

Pesticide poisoning with six organophosphorus compounds and a carbamate (carbofuran) was attributed to 41 out of 87 wild bird mortalities in Korea from 1998 to 2000. Waterfowls such as mallards (*Anas platyrhyncos*), mandrin ducks (*Aix galericuulata*) and white-fronted goose (*Anser albifrons*) were the most frequently affected group (Kwon *et al.*, 2004).

2.4 RESIDUE LEVELS OF CARBOFURAN

2.4.1 Environment

Williams *et al.* (1976) observed a build up of carbofuran residues in Fraser valley soils, Canada, after two years of pesticide application.

In a study designed to evaluate human exposure to carbofuran following aerial applications, it was estimated that the maximum inhaled doses were in the range of 0.7 to 2.0 mg/day (Draper *et al.*, 1981).

Carbofuran was detected only once (3 µg/l) in 678 samples from surveys of Canadian municipal and private water supplies conducted from 1971 to 1980 (Health and Welfare Canada, 1991).

Szeto and Price (1991) reported the persistence of carbofuran and fensulfothion at concentrations of 78 and 92 µg/g, respectively in Fraser Delta, Canada silt loam soils, almost a year after application.

Kimbrough and Litke (1996) reported a maximum carbofuran concentration of 1 µg/l in streams of U.S.A while the level of carbofuran in ground water ranged from 1 to 30 µg/l (WHO, 1996a).

The level of 3-hydroxy carbofuran was 18 µg/l in a surface water sample from an agricultural zone in Northwest Mexico when determined by liquid chromatography with post column fluorescence detection (Garcia and Bernal, 2001).

2.4.2 Residues in Crops and Fodder

The maximum residue limit (MRL) of carbofuran for food grains, milled food grains, fruits and vegetables, oil seeds and sugarcane is set as 0.10, 0.03, 0.10, 0.10, 0.10 ppm, respectively (PFA, 1997).

In a field trial conducted in Assam, carbofuran was applied with maize at 0.6kg active ingredient/ha in the seed furrows and the terminal residues in grain

and straw at harvest were 0.022 and 0.095 ppm, respectively (Goswami *et al.*, 2000).

Chakravarthy *et al.* (2000) reported that neither carbofuran nor its metabolites could be detected in harvested straw and grain samples of maize, which was treated with carbofuran at 1.0 and 2.0 kg active ingredient/ha during sowing.

In supervised field trials conducted in Brazil with soil application, residues of carbofuran in rice grains were < 0.01 mg/kg. In India, field trials with carbosulfan gave residues < 0.01 to 0.02 mg/kg in rice grains. Carbofuran residue of 0.06 mg/kg was detected in 1.7 per cent of rice samples from Korea in 1995. The residues in rice straw resulting from supervised trials of foliar and seed treatment application of carbosulfan in India were in the range of 0.01 to 0.03 mg/kg (FAO, 2004).

2.4.3 Residues in Animals

Avian gastro-intestinal tract contained carbofuran residues from non-detectable level to 640 µg/g (median 6.3 µg/g) in the wild bird kill accidents during the period from 1984 to 1988 resulting from the use of carbofuran on rice in California (Littrell, 1988).

Hunt *et al.* (1995) reported that crayfish (*Procambarus clarkii*), identified in the crop of herons that were exposed to carbofuran poisoning, contained residues of carbofuran up to 0.6 ppm, providing an additional evidence of exposure.

The crop content of a bald eagle and a red tailed hawk that were affected by pesticide poisoning, was mainly duck parts and on HPLC analysis carbofuran was detected at concentrations of 200 µg/g and 2.2 µg/g, respectively (Elliot *et al.*, 1996).

Metabolism studies in cow showed the residues of carbofuran in liver, kidney, muscle and fat. In goat the residues were detected in kidney, milk, fat and tissues (FAO, 2004).

2.5 ANALYSIS OF RESIDUES IN ANIMAL TISSUES

2.5.1 Sample Collection

Codex Alimentarius Commission has recommended guidelines (CAC/GL-33-1999) and methods for sampling for the detection of pesticide residues for compliance with MRL. The objective of the sampling procedures is to enable a representative sample to be taken from a lot for analysis, to determine compliance with Codex MRL for pesticides. Accordingly, MRL for pesticide residues in meat and poultry takes into account the maximum level expected to occur in the tissues of individual treated animals or birds. The MRL for meat and poultry apply to a bulk sample derived from a single primary sample (CAC, 1999).

For large mammals (cattle), minimum 500 g of whole/part of diaphragm supplemented by cervical muscles constitutes the laboratory sample for meat while for fat, 500 g (minimum) abdominal, subcutaneous or kidney fat from one animal forms a sample. The required minimum sample size for edible offals is 400 g (liver) and 200 g (kidney). For poultry meat (medium sized carcass of 500 g to 2 kg), 500g of thigh, leg or other dark meat from at least 3 birds constitute the sample for residue analysis while a minimum of 500 g abdominal fat from at least 3 birds is the requirement for poultry fat. In case of edible bird offals, units from at least 6 birds (minimum 200 g) form the required sample size. In goose and duck liver and similar high value products, it is unit of minimum 50 g from one bird (CAC, 1999).

The laboratory sample must be placed in a clean inert container, which provides secure protection from contamination, damage and leakage. The container should be sealed, securely labeled and the samples of meat and poultry

should be frozen prior to despatch, unless transported to the laboratory before spoilage can occur (CAC, 1999).

2.5.2 High Performance Liquid Chromatography (HPLC)

In HPLC analysis of pesticide residues, a commercial reverse phase column, C-18 bonded to silica will separate pesticides and can be regenerated with pure solvent washes or the use of guard column. The advantage of reverse phase chromatography is that the polar solvents used such as methanol and acetonitrile diluted with water enable the cleaning of delicate reciprocal pumps. These pumps generate pressure as high as 6000 psi (Zweig, 1984).

The HPLC system comprises of high pressure pumps, guard column, columns packed with microparticulate phases and a detector that continuously record the concentration of the sample. In residue analysis either, UV or fluorescence or electrochemical detectors are used. A mobile phase causes component of pesticide mixture to move through the stationary phase in the column and to separate from one another. Differential migration occurs because of differences in distribution between two phases (McMahon and Wagner, 1989).

A liquid chromatographic (LC) method was developed by Ali (1989) using a 2-step purification technique for the simultaneous determination of ten carbamates in bovine, swine and duck livers. Bakowski *et al.* (1994) developed a gas chromatographic mass spectrometric (GC-MS) confirmation procedure for the determination of N-methyl carbamate residues in liver tissues.

The HPLC analysis of N-methyl carbamates was carried out under reverse phase condition using octadoceyl, octyl silica columns and mixture of water and organic solvents as mobile phase (Yang *et al.*, 1996).

Kawamoto and Makihata (2003) reported that carbamate pesticides thermally decompose at the gas chromatography injection port and HPLC detection is a more suitable method for their analysis.

2.5.2.1 ***Extraction and Clean up***

The N-methyl carbamate residues from liver samples of bovine, swine and duck were extracted with methylene chloride. After evaporation, the residues from the extract were dissolved in methylene chloride-cyclohexane (1+1), clean up done by gel permeation chromatography and further purified the residues by passing through an aminopropyl Bond Elut extraction cartridge (Ali, 1989).

The analytical method for determination of residues of carbofuran in beef involved extraction with acetone followed by centrifugation, liquid-liquid partition and clean up for reverse phase HPLC analysis (Chen, 1995). He also extracted carbofuran and 3-hydroxy carbofuran metabolites from beef and poultry meat after initial acid hydrolysis of the matrix, clean up by liquid-liquid partition, redissolved in acetonitrile and analysed using reverse phase HPLC.

An extraction method for the analysis of residues of carbofuran in meat, milk and egg which includes initial extraction with hexane/acetonitrile (4:1 v/v) mixture, partitioning into acetonitrile and clean up with solid phase extraction for HPLC analysis was developed by Ginzburg (2001).

Liu *et al.* (2001) described a new method to determine simultaneously the amount of furathiocarb and metabolites: carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran in biological tissues such as liver and kidney which consists of extraction of samples with acetone, filtration, partition, purification of target analytes through a silica gel column and HPLC determination with post-column derivatisation.

2.5.2.2 *Detection*

The pesticide carbamate has an aromatic moiety and U.V absorbing group and therefore fixed/variable wavelength UV detector for HPLC is applicable (Zweig, 1984).

Ali (1989) analysed N-methyl carbamate residues in liver samples of bovine, swine and duck by liquid chromatography using post-column derivatisation with ortho-phathaladehyde and fluorescence detection at excitation and emission wavelengths of 340 and 418 nm, respectively.

Aaron and Coly (2000) opined that HPLC with UV-visible absorption detectors was the technique of choice for residue screening works, especially for thermally labile pesticides like carbamates.

2.5.2.3 *Recovery Studies*

Beef, pork and duck liver samples were fortified with 5,10 and 20 ppb of mixed carbamate pesticide standards and the average recovery at all three levels of fortification was greater than 80 per cent with coefficient of variation less than 17 per cent (Ali, 1989).

Holstege *et al.* (1994) described a multiresidue method for the detection of organophosphorus, organochlorine and N-methyl carbamate residues in plant and animal tissues and the analysis of fortified bovine liver samples resulted in an average recovery of 96 ± 4 per cent at 0.5 to 0.05 µg/g fortification levels. The method detection limits ranged from 0.02 to 0.5 µg/g for the compounds with a 10g sample.

Reasonable recoveries obtained for routine analysis with the limits of detection (LOD) in fluorescence detection as 0.2, 0.1, 0.1 and 0.2 mg/l for furathiocarb, carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran, respectively in a HPLC method developed by Liu *et al.* (2001).

The recovery of carbofuran spiked in beef at 0.05 and 0.50ppm levels was 90.8 per cent and 100.5 per cent, respectively (Ginzburg, 2001). The recovery of carbosulfan from beef was 74.4 per cent and 78.3 per cent at the same fortification levels.

2.5.3 Maximum Residue Limits (MRL)

The tolerance limit of carbofuran for meat and poultry on carcass fat basis was recommended as 0.1 mg/kg (PFA, 1997; MFPO, 1973).

The residues of pesticide in samples of vegetarian and nonvegetarian diets collected from different hotels in six districts of Andhra Pradesh were monitored during 1993-94 and observed that hexachlorocyclohexane residues were above MRL, while those of DDT, aldrin, dieldrin and pyrethroids were below MRL. However, carbaryl and carbofuran were not detected in diets when analysed by spectrophotometer method (Reddy *et al.*, 2000).

The residue is defined in compliance with MRL as the sum of carbofuran and 3-hydroxy carbofuran, expressed as carbofuran. In India the MRL of carbofuran for all commodities is set at 0.1 mg/kg (FAO, 2004).

Materials and Methods

MATERIALS AND METHODS

The research work was undertaken to develop and standardise a suitable method of extraction of residues of carbofuran and its metabolites from meat, liver, kidney and fat of buffaloes and ducks for High Performance Liquid Chromatographic analysis and to apply the method in the determination of the residues in these tissues of buffaloes and ducks.

3.1 SAMPLE COLLECTION AND PREPARATION

3.1.1 Sampling

Samples were collected according to the Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs of Codex Alimentarius Commission (CAC, 1999). Fifteen samples each of various tissues from buffaloes (*Bubalus bubalis*) and ducks (*Anas platyrhynchos*), viz., muscle, liver, kidney and fat were collected at random from different lots of animals slaughtered at different parts of Kerala including those from the Department of Livestock Products Technology, College of Veterinary & Animal Sciences, Mannuthy. Each tissue sample composed of 500 g each of diaphragm and kidney fat, 400 g liver and 200 g kidney from each buffalo constituted a sample. In the case of ducks, pooled samples of each tissue were collected at random from 10 birds in a lot of over 100 to form a single representative sample. Not less than 50 g each of thigh meat and abdominal fat, 20 g kidney and 50 g liver from each duck was collected and pooled to form a representative sample of a lot. Fifteen such samples were taken from ducks. The samples in labeled High Density Polyethylene pouches were transported to the laboratory in insulated containers and stored at -20° C till further treatments.

3.1.2 Initial preparation of the Samples

All the glasswares used in the residue analysis were cleaned thoroughly and rinsed with deionised double distilled water followed by Nanopure water (Nanopure Diamond, Barnstead, U.K). The dried glasswares were rinsed again with the respective solvents before use.

All the chemicals and water used were of HPLC grade (Merck Ltd, India) For the initial preparation, 2 g minced sample of each tissue from buffaloes and ducks were taken in duplicate boiling tubes containing 0.5 g anhydrous sodium sulphate (Na_2SO_4).

3.2 EXTRACTION METHOD

Different solvents, viz., acetonitrile, acetone, methanol, hexane and 2-propanol were utilised for the extraction of carbofuran residues and its metabolite from different tissues. From the trial the best suitable solvent was chosen and used for the extraction studies.

3.2.1 Extraction with acetonitrile

To the boiling tube containing the tissue and anhydrous Na_2SO_4, ten ml of acetonitrile (ACN) was added and homogenised at 9000 rpm for 5 min. in a Polytron Tissue Homogeniser PT 3100 (Kinematica, Switzerland). The supernatant was filtered into a conical flask through a filter bed of anhydrous Na_2SO_4 in a funnel. The homogenisation was repeated twice at 9000 rpm for 3 min. followed by filtration as before. The solvent volume in the conical flask was reduced to 10 ml in a Rotary Vacuum Evaporator (Butchy type) at 40-50° C. The conical flask was covered with parafilm and kept for further cleanup.

3.2.2 Extraction with acetone, methanol, 2-propanol and hexane

The above extraction method was repeated for meat, liver, kidney and fat using each solvent, viz., acetone, methanol, 2-propanol and hexane. The final volume was reduced to dryness in a Rotary Vacuum Evaporator in the case of extraction with acetone, methanol and 2-propanol. The residue in the evaporator flask was redissolved in 10 ml ACN. For extraction with hexane, the final volume of the solvent was reduced to 10 ml in the evaporator flask. The extracts were transferred to conical flasks and covered with parafilm for further cleanup.

3.3 CLEANUP

The cleanup of the extracted samples from each tissue using different solvents was done by liquid-liquid partitioning with ACN and hexane.

3.3.1 Meat, Kidney and Liver

The extracted sample in 10 ml ACN was transferred to a 125 ml separating funnel together with 5 ml hexane, stoppered and shook for 5 min with intermittent air release. The bottom ACN layer was collected in a conical flask. Five ml of ACN was again added to the hexane layer in the separating funnel and shook for 3 min. The bottom layer was collected to the previous conical flask and the hexane layer was discarded. The total 15 ml ACN fraction saved in the conical flask was again partitioned twice with hexane as before. The final ACN fraction was filtered through a bed of anhydrous Na_2SO_4 into a conical flask.

3.3.2 Fat

The extract of fat in hexane was taken in a separating funnel together with 5ml ACN and shook well for 5 min. Then the bottom layer was collected into a conical flask. Again added 10ml ACN to the separating funnel and shook for

3 min. The bottom layer was collected in the previous conical flask and the hexane layer was discarded. This fraction in the conical flask was again partitioned twice with hexane as before. The final ACN fraction was filtered through a bed of anhydrous Na_2SO_4 into a conical flask.

After cleanup the ACN fractions of 15 ml each of all the tissues were evaporated to dryness in a Rotary Vacuum Evaporator. The residues were redissolved in 1 to 2 ml of acetonitrile and transferred to 5 ml screw capped glass vials for refrigerated storage until HPLC analysis.

3.4 HPLC ANALYSIS

All the samples were purified before chromatographic analysis by filtering through disposable cartridges with 0.2 μ cellulose filters (Spartan 13/0, 2 RC, Schleicher and Schnell, Germany). All the solvents used in HPLC were vacuum filtered through 0.45μ membrane filter (NL 17, Schleicher and Schnell, Germany) in an all glass vacuum filtration assembly (Riviera, India).

Details of HPLC system used:

System: Schimadzu LC- 10 AVP series (Shimadzu Corporation, Kyoto, Japan)
Injector: Manual injection with Hamilton Syringe (25 μl)
Column: Reversed phase Luna 5μ, C18 (2) Column (Phenomenex, USA)
Detector: variable wavelength UV/VIS detector installed with Class VP Software
Pump: Binary gradient

Operational Parameters:

The mobile phase - ACN/water - ratio and flow rate were optimised by trial and error method. The total run time chosen for analysis was 25 min. by trial and error.

Mobile Phase: Acetonitrile: water (35:65)

Flow rate: 0.7-1.3ml/min. (programmed)

Sample volume: 20 µl

Column temperature: Ambient

Detector Wavelength: 211nm

3.5 STANDARDISATION

Certified standard references of carbofuran and its metabolites, viz., 3-hydroxy carbofuran, 3-keto carbofuran, 3-hydroxy 7-phenol carbofuran, 3-keto 7-phenol carbofuran and carbofuran phenol used for the study were of Dr.Ehrenstorfer Gmbh, Augsburg, Germany. The standards were dissolved in ACN at different concentrations. The samples of above standards were analysed in HPLC to identify the chromatogram peaks of respective compounds.

3.5.1 Linearity study

The linearity of the reference standard compound carbofuran and its metabolite 3-hydroxy carbofuran was assessed by injecting the compound in acetonitrile (20µl) at 1, 10 and 100 ppm levels into the manual injector port of HPLC using Hamilton syringe. The area of the peaks in the chromatogram was plotted against graded concentration of carbofuran and hydrocarbofuran to assess the linearity of the curve. The concentration of residue per sample was calculated in ppm using the equation:

Amount of residues (ppm) = X x V/W, where

X = chromatogram reading in ppm

V = total volume of the cleaned up sample

W = weight of the sample taken for extraction.

3.5.2 Recovery Study

In order to assess the percentage recovery of carbofuran by different solvents, buffalo tissues, viz., meat, liver, kidney and fat were spiked with 100 ppm carbofuran standard. Then the residue was extracted with different solvents, viz., ACN, acetone, methanol, 2-propanol and hexane for clean up and further analysis in HPLC. 20 μl of each cleaned up sample was injected into HPLC using 25 μl Hamilton syringe and the residue was estimated in comparison with the reference standard. The best solvent was selected on the basis of the highest percentage of recovery of standard carbofuran. The suitability of the selected solvent was reassessed on the basis of recovery rate after fortifying different tissues of buffalo with 1, 10 and 100 ppm carbofuran standard.

3.6 SAMPLE SCREENING

The selected solvent system was applied for the extraction of residues of carbofuran and its metabolites in a further HPLC residue screening programme in the collected tissue samples of buffaloes and ducks. The buffalo matrices used for fortification and recovery study were also screened for any possible presence of these residues.

3.7 STATISTICAL ANALYSIS

The results obtained were analysed with suitable tools (Snedecor and Cochran, 1994) and comparatively analysed with respect to the Maximum Residue Limit.

Results

RESULTS

The research work was undertaken with the objective of developing and standardising a suitable method of extraction of carbofuran and its metabolite from meat, liver, kidney and fat of buffaloes and ducks for HPLC analysis and to apply the method in the determination the residues in these tissues. The results are furnished in the following sections.

4.1 Standardisation of HPLC conditions

The mobile phase and flow rate were optimised by trial and error method. When acetonitrile alone was used as the mobile phase at a flow rate of 1ml/min. the compound 3-hydroxy carbofuran did not elute within 20 min. The polarity of the mobile phase was increased by adding water to ACN at a ratio of 75:25 (ACN/water) and the compound eluted at 14.6 min. When the ratio was 50:50, the elution time reduced to 10.8 min. On further increasing the concentration of water to a ratio of ACN/water 35: 65 an elution time of 5.542 min. was obtained at a flow rate of 1ml/min as illustrated in Figure 1.

Similarly, for carbofuran there was no elution within 20 min. when the mobile phase was acetonitrile 100 per cent, ACN/water 75: 25 or ACN/water 50:50. The compound eluted at 17.258 min. when the mobile phase was ACN/water 35: 65 as shown in Figure. 2. In order to reduce the retention time, the flow rate was increased to 1.3ml/min. and found that the compound eluted at 14.058 min. (Fig. 3).

In order to optimise the elution of 3-hydroxy carbofuran, carbofuran and other metabolites within 20 min., the flow rate was time programmed as follows:

0.7 ml/min. - 0 min.

1.0 ml/min. - 11 min.

1.2 ml/min. - 14 min.

1.3 ml/min. - 17 min.

The compounds 3-hydroxy carbofuran and carbofuran eluted at 6.883min. and 18.383 min., respectively at this flow rate for a total 25 min. run time is illustrated in Figures 4 and 5.

The chromatograms of other metabolites, viz., 3-hydroxy-7-phenol carbofuran, 3-ketocarbofuran, 3-keto-7-phenol carbofuran and carbofuran phenol are presented in Figures 6 to 8 in which the retention times are 4.275 min., 7.8 min., 8.308 min. and 19.925 min., respectively.

4.2 Linearity Study

The linearity of the reference standard compound carbofuran and its metabolite 3-hydroxy carbofuran was assessed by plotting a graph of the concentration (ppm) against area under the respective chromatogram peaks. The peak area of known concentrations of carbofuran and 3-hydroxy carbofuran in ACN is given in Table 1 and 2, respectively.

The peak areas of carbofuran in the chromatogram at the concentration levels of 1, 10, and 100 ppm were 0.657×10^5, 6.824×10^5 and 54.13×10^5, respectively. In the case of 3-hydroxy carbofuran the areas in the chromatogram were 0.808×10^5, 8.073×10^5 and 80.069×10^5 for concentrations 1, 10 and 100 ppm, respectively.

The results showed excellent linearity for the reference standards under the set operating conditions as illustrated in Figures 9 and 10.

4.3 Recovery Study

The percentage recovery of carbofuran from buffalo tissues was assessed after fortifying them with the certified standard at 100 ppm. Different solvents were employed for extraction and the recovery rate (%) of carbofuran at 100 ppm fortification level using each solvent is given in Table: 3.

The recovery of carbofuran from buffalo meat using ACN was in the range of 89 to 95 per cent. The recovery from other tissues ranged from 87 to 91, 89 to 91 and 56 to 58 per cent, respectively for liver, kidney and fat.

The recovery of acetone extracted carbofuran from meat, liver, kidney and fat were 39 to 42, 45 to 48, 41 to 43 and 36 to 40 per cent, respectively.

When methanol was used for extraction, the respective recovery of carbofuran from meat, liver, kidney and fat were 2 to 5, 10 to 12, 7 to 9 and 14 to 16 per cent

For the extraction method using 2-propanol, the recovery of carbofuran was 6 to 8, 4 to 10, 9 to 11 and 13 to 15 per cent, respectively from meat, liver, kidney and fat.

The recovery of hexane extracted carbofuran from meat, liver, kidney and fat were 50 to 56, 46 to 51, 49 to 53 and 86 to 93 per cent, respectively.

The highest percentage of recovery of carbofuran from fortified buffalo meat, liver and kidney was obtained on extraction with ACN. While for fat hexane was found to be the best solvent for extraction of carbofuran.

4.4 Solvent selection

Based on the recovery, ACN was selected as the best solvent for extraction of residues of carbofuran from meat, liver and kidney. For the extraction of residues from fat, hexane was selected as the best solvent based the recovery rate. This solvent system was applied in the recovery study of carbofuran from buffalo tissues at different fortification levels to assess its suitability for recovery of lower concentrations. The details are presented in Table 4.

The mean recovery percentage of carbofuran from meat, liver, kidney and fat were 87.4 ± 2.01, 85.4 ± 1.80, 89.1 ± 1.90 and 82.0 ± 1.04, respectively at 1 ppm fortification level. At 10 ppm fortification level the recovery rates were 90.3 ± 1.98, 87.5 ± 2.01, 88.9 ± 2.44 and 86.3 ± 1.72 for meat, liver, kidney and fat respectively. The mean recovery percentage at 100 ppm fortification level were 92.5 ± 2.57, 89.2 ± 2.86, 90.6 ± 3.01 and 89.5 ± 1.95 for meat, liver, kidney and fat.

The chromatogram showing the recovery of carbofuran from buffalo meat after fortification and extraction with ACN is shown in Fig.11.

The buffalo matrices - meat, liver, kidney and fat - used for carbofuran fortification and recovery study were also screened for any possible presence of residues. No residues could be detected in these samples as shown in Fig. 12 to 15.

4.5 Sample screening

The method developed for the extraction of carbofuran and its metabolite was applied in the screening of buffalo and duck samples collected from different parts of Kerala. The results are shown in Tables 5 and 6.

The buffalo tissue samples screened did not show the presence of the residues of carbofuran and its metabolites, viz., 3-hydroxy carbofuran, 3-keto carbofuran, 3-hydroxy 7-phenol carbofuran, 3-keto 7-phenol carbofuran and carbofuran phenol in detectable amounts by the present method of HPLC analysis. Therefore, these were designated as Below Detection Limit (BDL).

The chromatogram of buffalo meat, liver, kidney and fat screened for residues of carbofuran and its metabolites is given in Fig. 16 to 19.

Except in one duck sample all other samples were BDL and the chromatogram of that is shown in Fig. 20 to 23. 3-hydroxy carbofuran was detected in meat and kidney at concentration of 2ppm and 0.9 ppm, respectively while no residues could be detected in liver and fat.

Table 1. Peak area values of known concentrations of carbofuran in acetonitrile on estimation by HPLC

Sl.No	Concentration (ppm)	Area
1	1	0.657×10^5
2	10	6.824×10^5
3	100	54.13×10^5

Table 2. Peak area values of known concentrations of 3-hydroxycarbofuran in acetonitrile on estimation by HPLC

Sl.No	Concentration(ppm)	Area
1	1	0.808×10^5
2	10	8.073×10^5
3	100	80.069×10^5

Table 3. Recovery rate (%) of carbofuran from different matrices using different solvents

Solvents \ Tissues	Meat	Liver	Kidney	Fat
Acetonitrile	89 – 95	87 – 91	89 – 91	56 - 58
Acetone	39 – 42	45 – 48	41- 43	36 -40
Hexane	50 – 56	46 – 51	49 – 53	86 – 93
Methanol	2 – 5	10 – 12	7 – 9	14 - 16
2-propanol	6 – 8	4 – 10	9 – 11	13-15

Table 4. Mean recovery (%) of carbofuran from different matrices on fortification with known concentrations (mean ± S.E)

Level of addition	Meat	Liver	Kidney	Fat
1 ppm	87.4 ± 2.01	85.4 ± 1.80	89.1 ± 1.90	82.0 ± 1.04
10 ppm	90.3 ± 1.98	87.5 ± 2.01	88.9 ± 2.44	86.3 ± 1.72
100 ppm	92.5 ± 2.57	89.2 ± 2.86	90.6 ± 3.01	89.5 ± 1.95

Table 5. Concentration of residues of carbofuran and its metabolites in buffalo samples

Compounds	Meat	Liver	Kidney	Fat
Carbofuran	BDL	BDL	BDL	BDL
3-hydroxy carbofuran	BDL	BDL	BDL	BDL
3-ketocarbofuran	BDL	BDL	BDL	BDL
3-OH-7-phenol carbofuran	BDL	BDL	BDL	BDL
3-keto-7-phenol carbofuran	BDL	BDL	BDL	BDL
Carbofuran phenol	BDL	BDL	BDL	BDL

(BDL- Below Detectable Limit)

Table 6. Concentration of residues of carbofuran and its metabolites in duck samples

Compounds	Meat	Liver	Kidney	Fat
Carbofuran	BDL	BDL	BDL	BDL
3-hydroxy carbofuran	BDL*	BDL	BDL*	BDL
3-ketocarbofuran	BDL	BDL	BDL	BDL
3-OH-7-phenol carbofuran	BDL	BDL	BDL	BDL
3-keto-7-phenol carbofuran	BDL	BDL	BDL	BDL
Carbofuran phenol	BDL	BDL	BDL	BDL

(* detected in one sample at 2ppm and 0.9 ppm in meat and kidney, respectively)

Figure.1 Chromatogram of 3-hydroxycarbofuran (100 ppm) at a flow rate of 1ml/min.

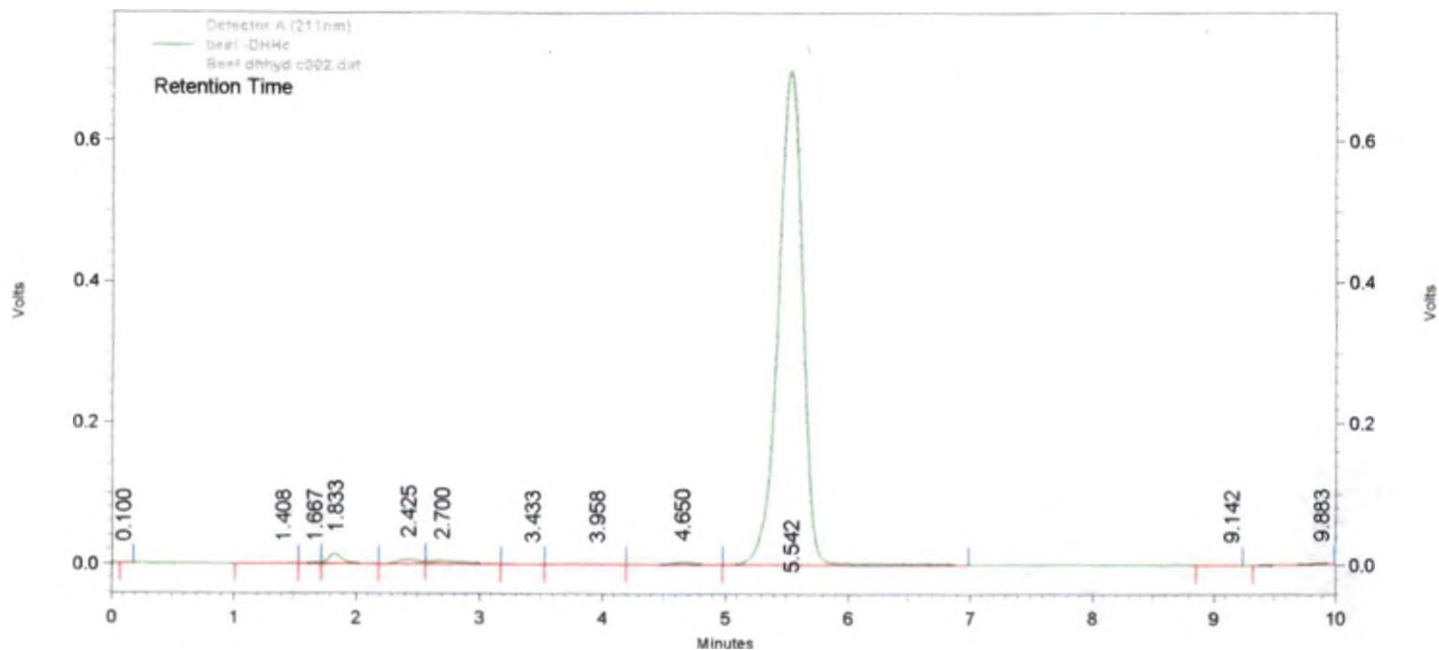


Figure.2 Chromatogram of carbofuran (10ppm) at a flow rate of 1ml/min.

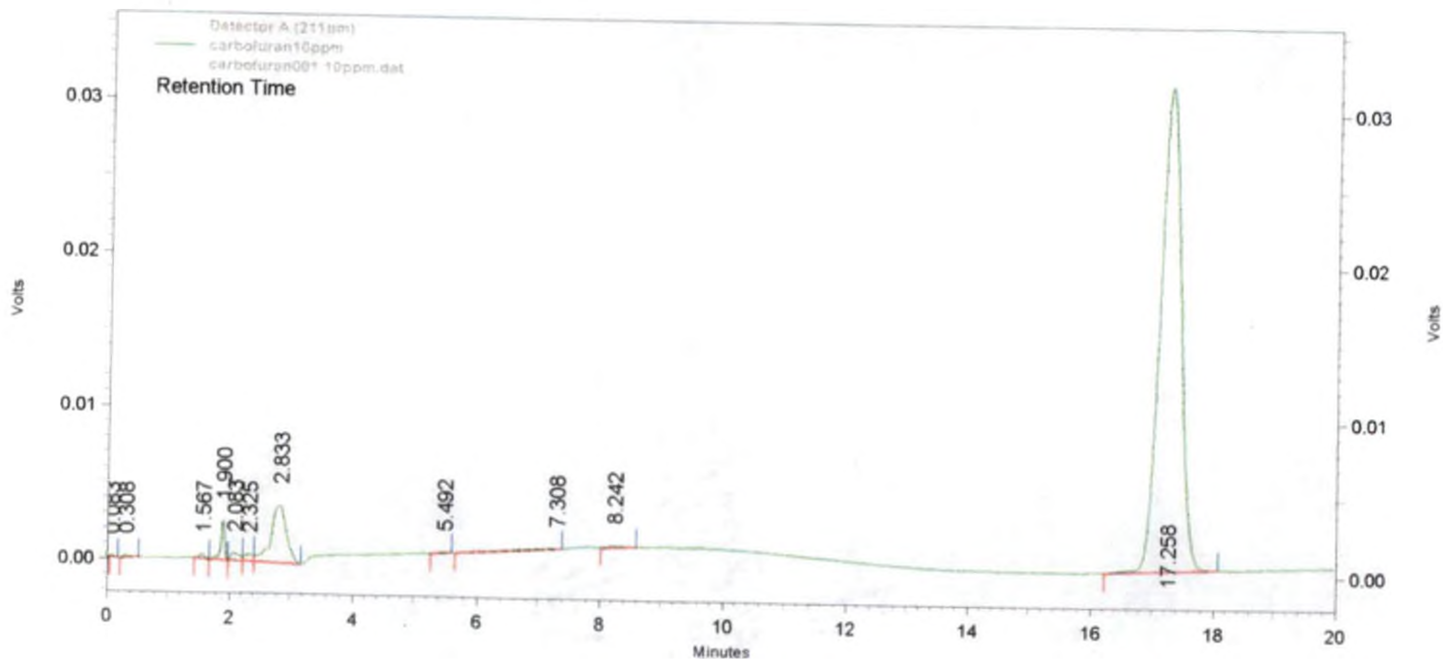


Figure.3 Chromatogram of carbofuran (100 ppm) at a flow rate of 1.3ml/min.

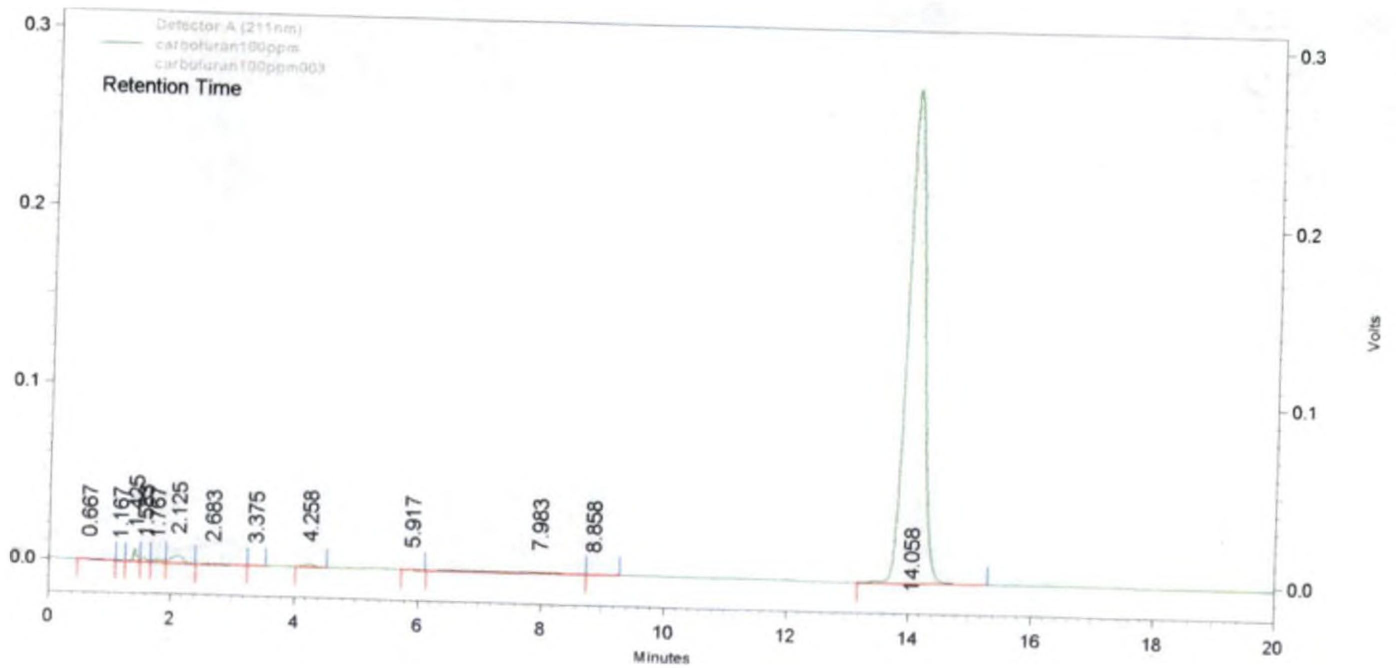
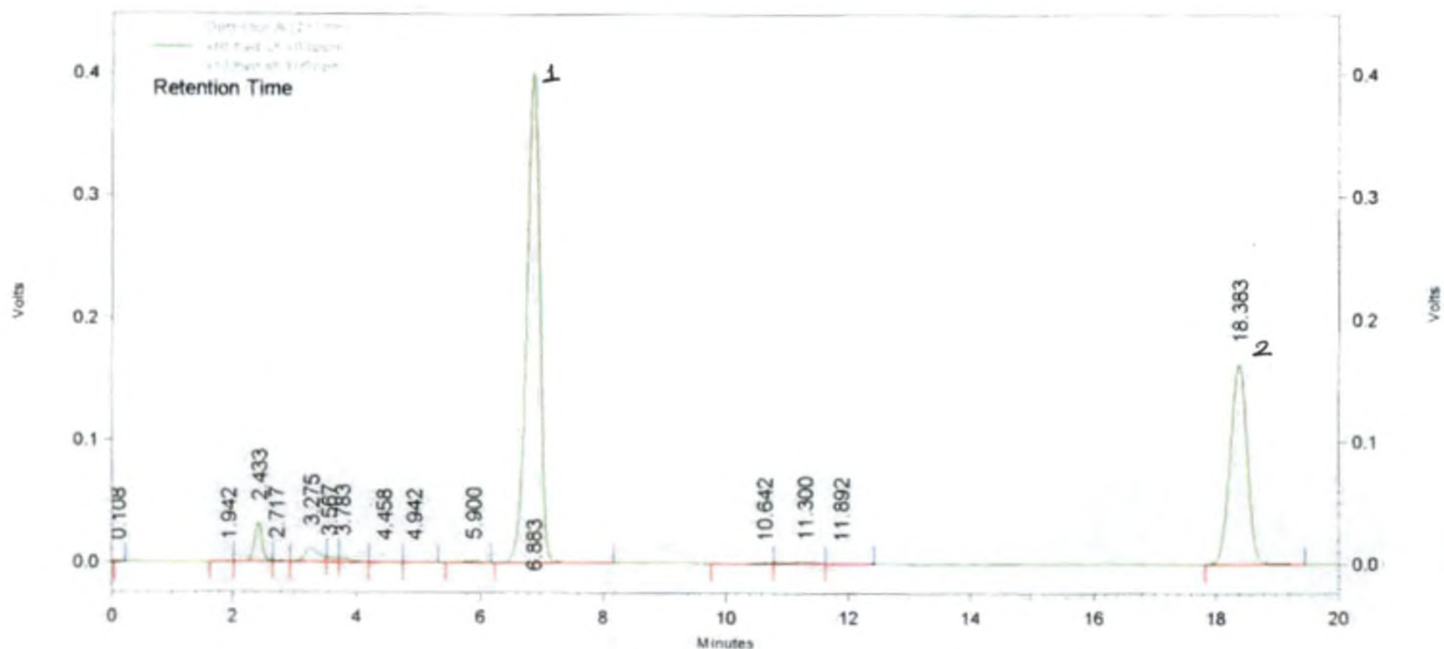


Figure.4 Chromatogram of carbofuran (100 ppm) and 3-hydroxycarbofuran (100 ppm) under programmed flow rate.



1 .3- Hydroxycarbofuran 2. Carbofuran

Figure.5 Chromatogram of carbofuran (100 ppm) at programmed flow rate.

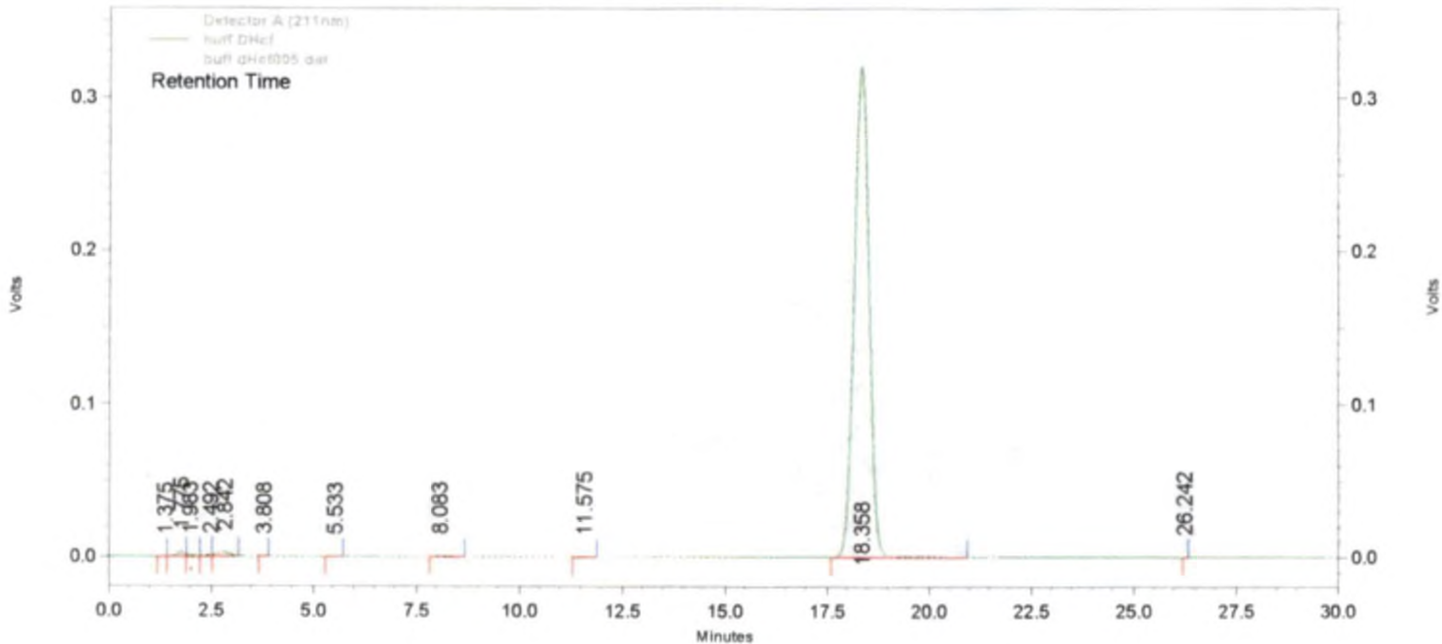
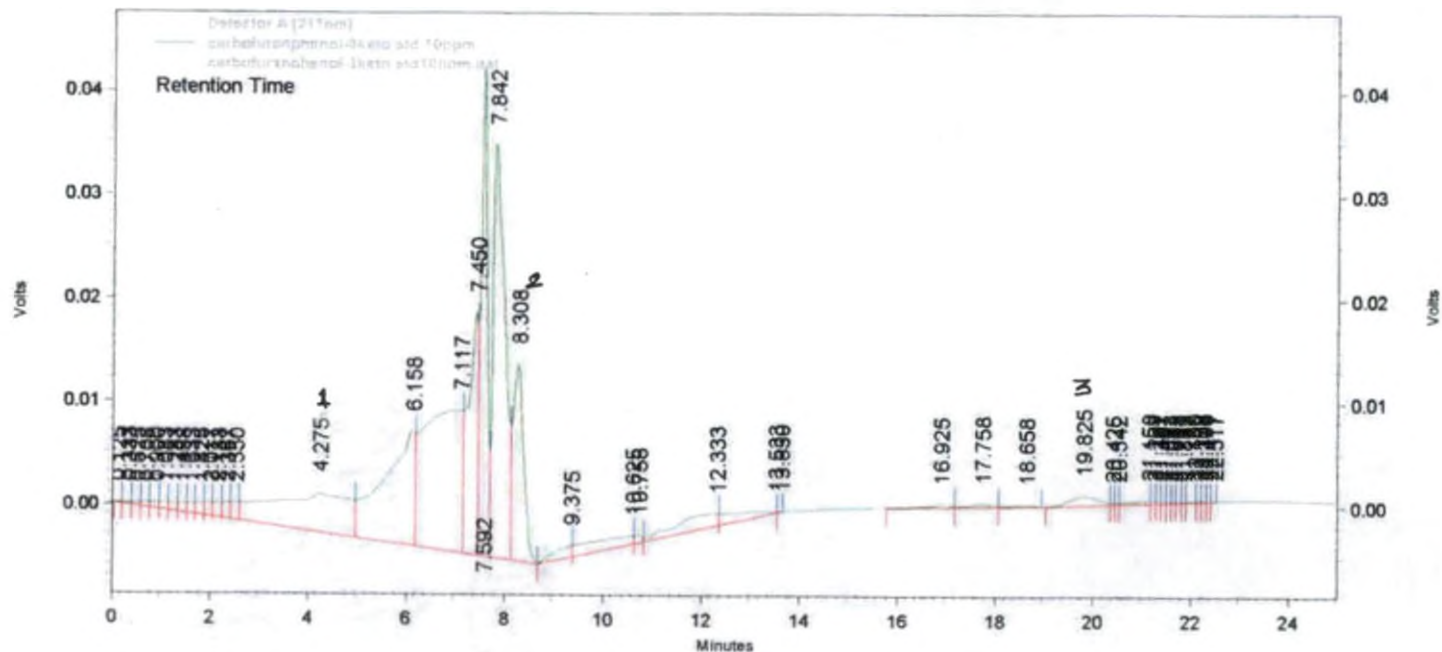
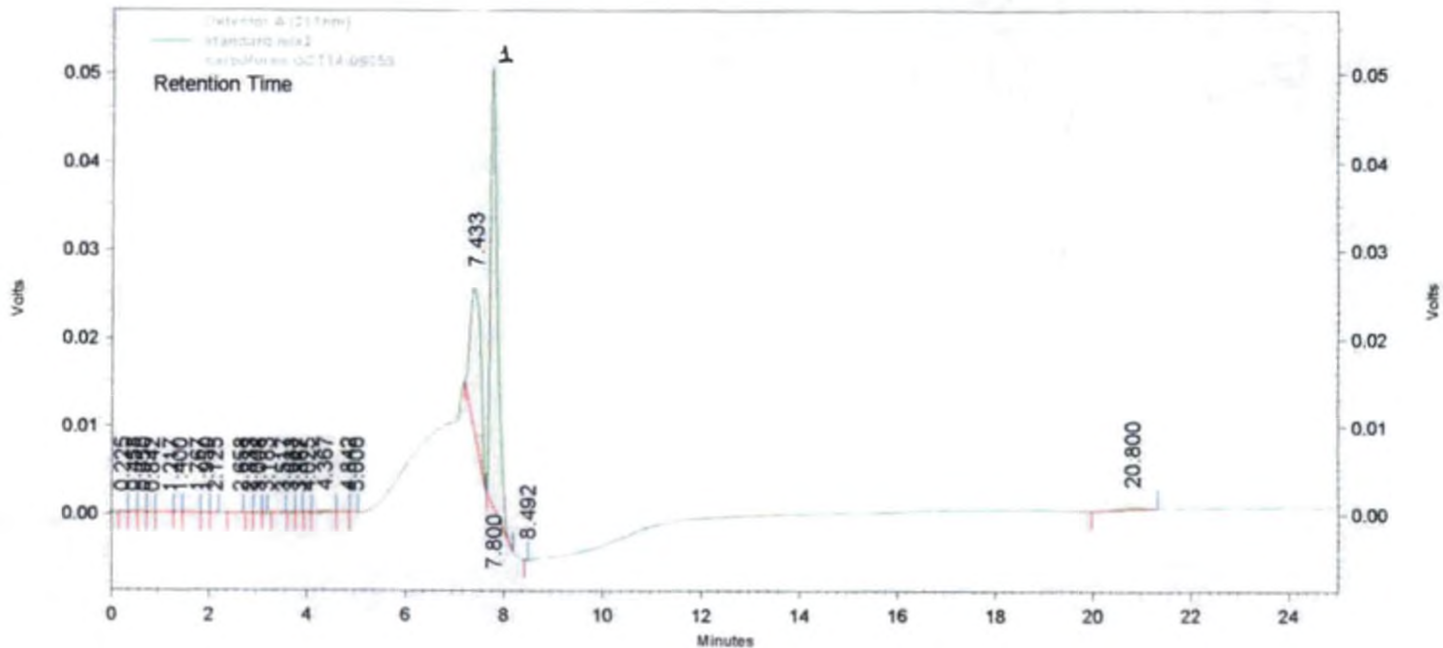


Figure.6 Chromatogram of 3-OH-7-phenol carbofuran, 3-keto-7-phenol carbofuran and carbofuran phenol



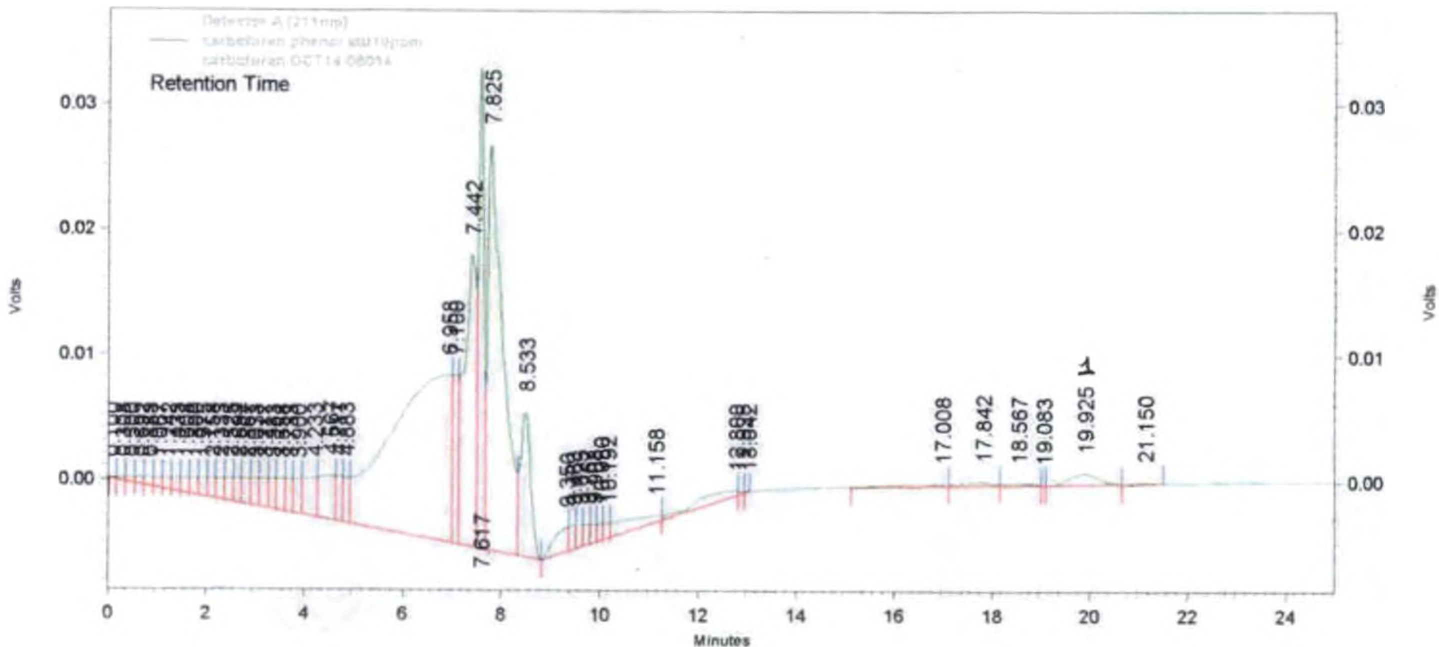
1. 3-OH-7-phenol carbofuran 2. 3-keto-7-phenol carbofuran 3. carbofuran phenol

Figure.7 Chromatogram of 3-keto carbofuran



1. 3-keto carbofuran

Figure.8 Chromatogram of carbofuran phenol



1. Carbofuran phenol

Figure. 9 Linearity of peak area values against known graded concentration of carbofuran

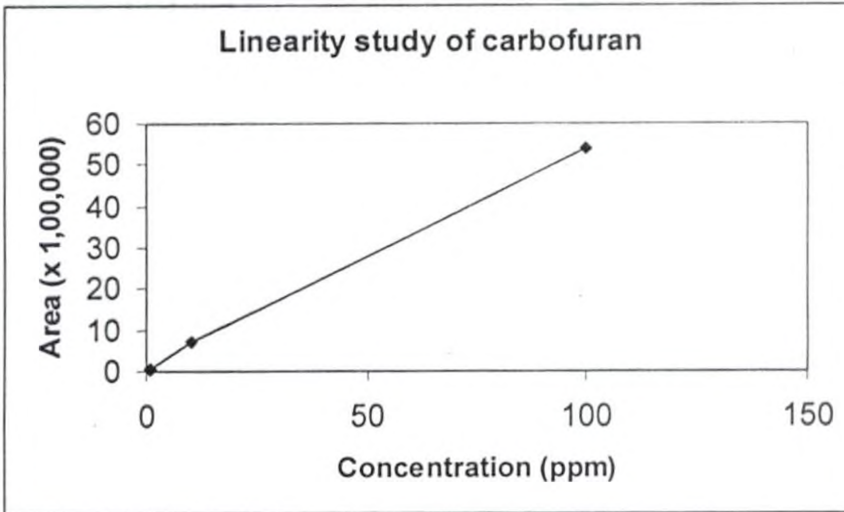


Figure. 10 Linearity of peak area values against known graded concentration of 3-hydroxycarbofuran

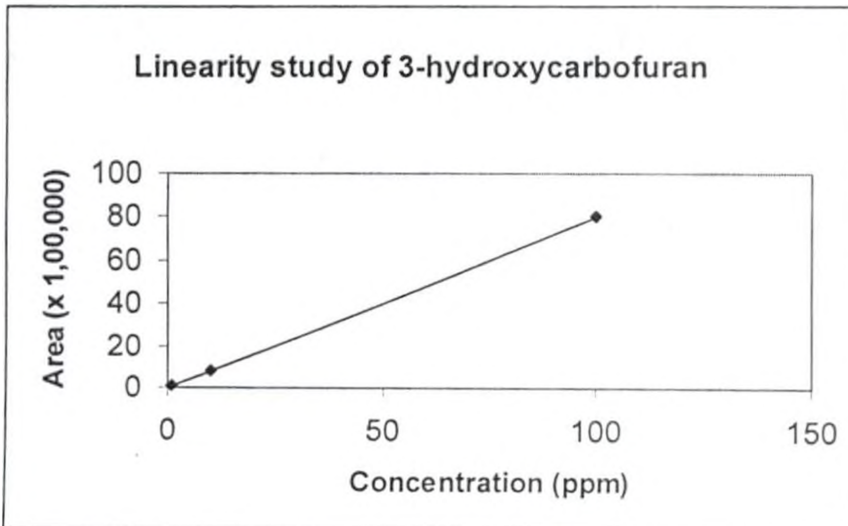


Figure.11 Recovery of carbofuran from buffalo meat after fortification and extraction with acetonitrile

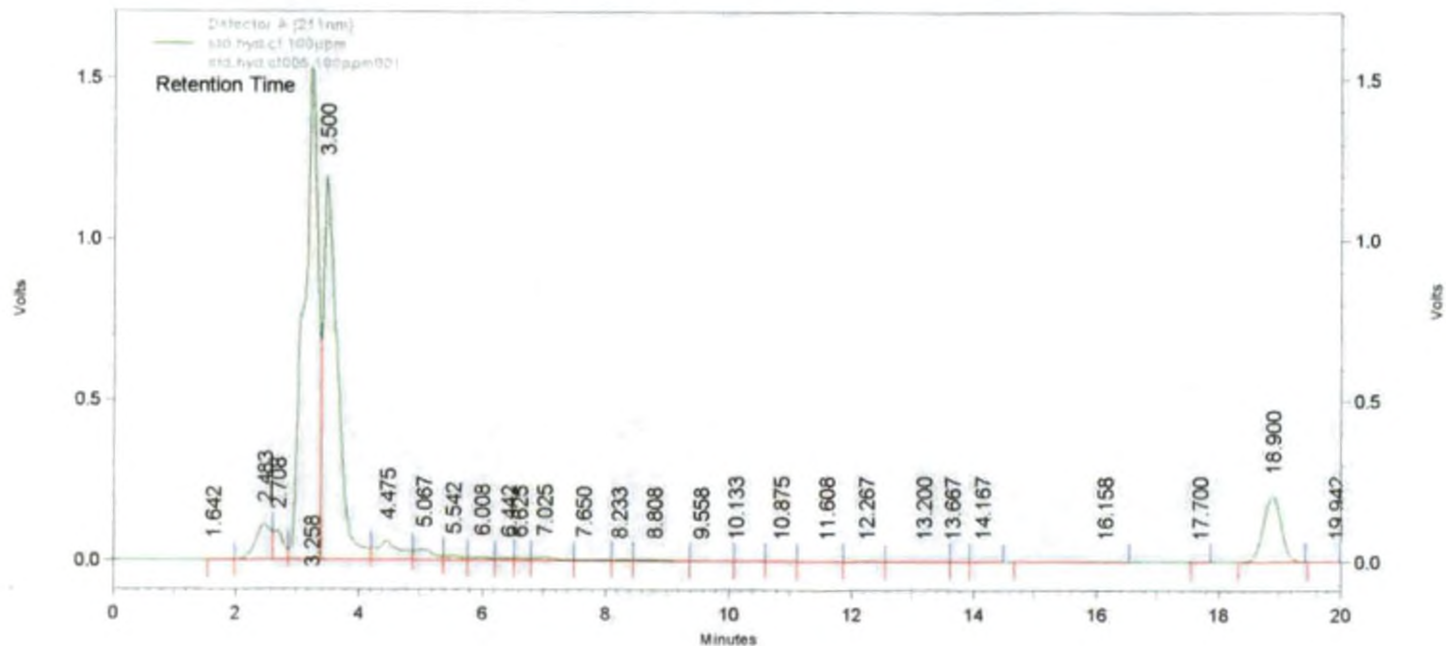


Figure.12 Chromatogram of buffalo meat used for carbofuran fortification and recovery study

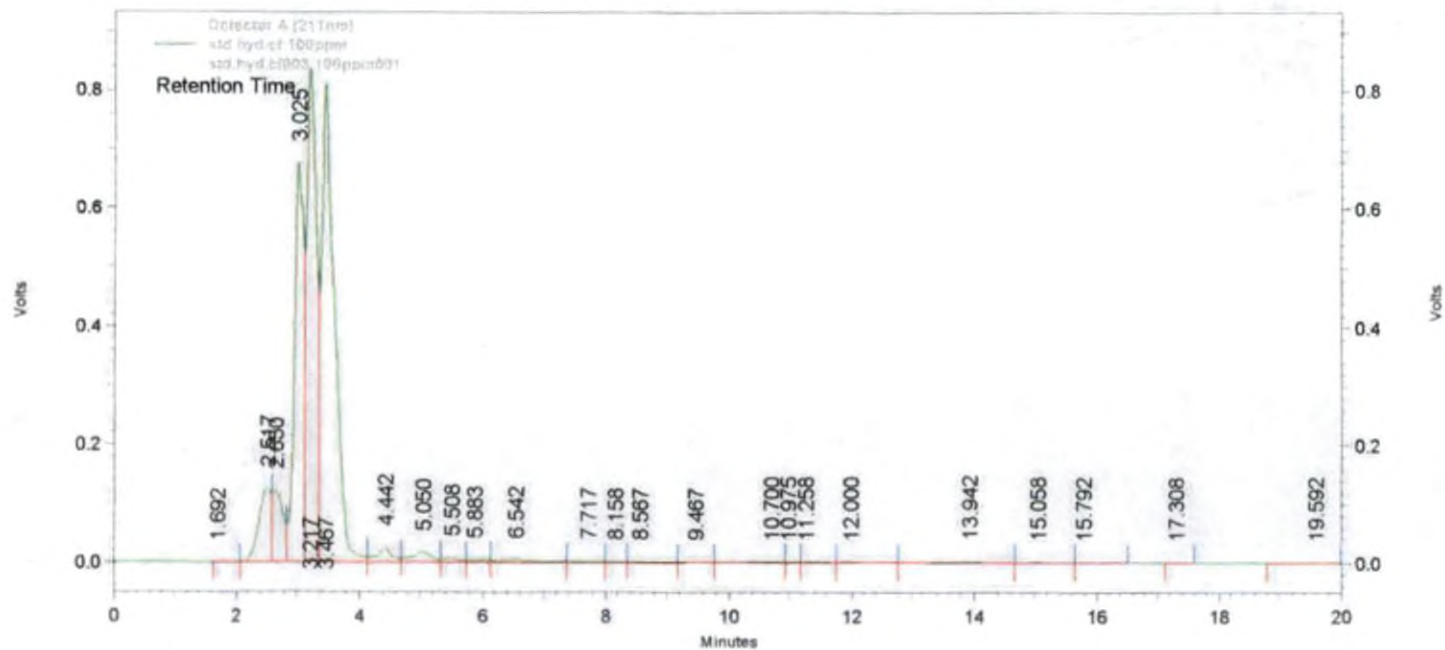


Figure.13 Chromatogram of buffalo liver used for carbofuran fortification and recovery study

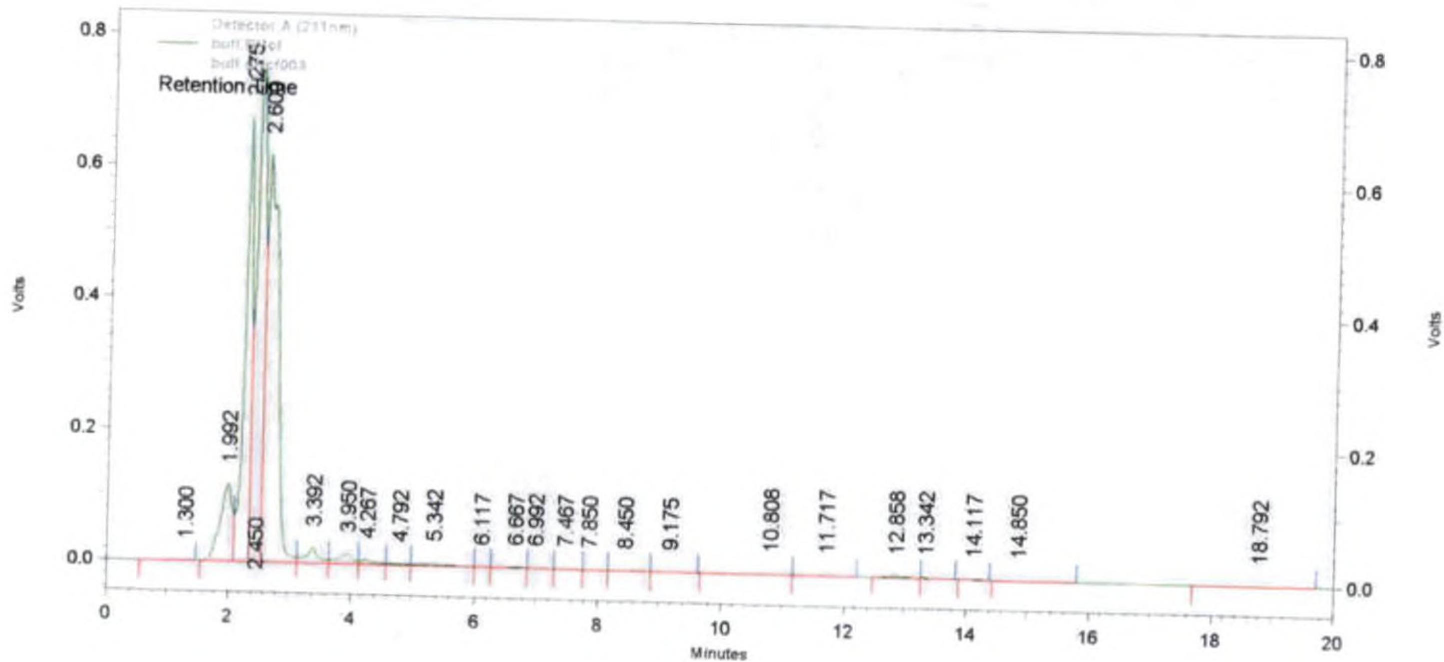


Figure.14 Chromatogram of buffalo kidney used for carbofuran fortification and recovery study

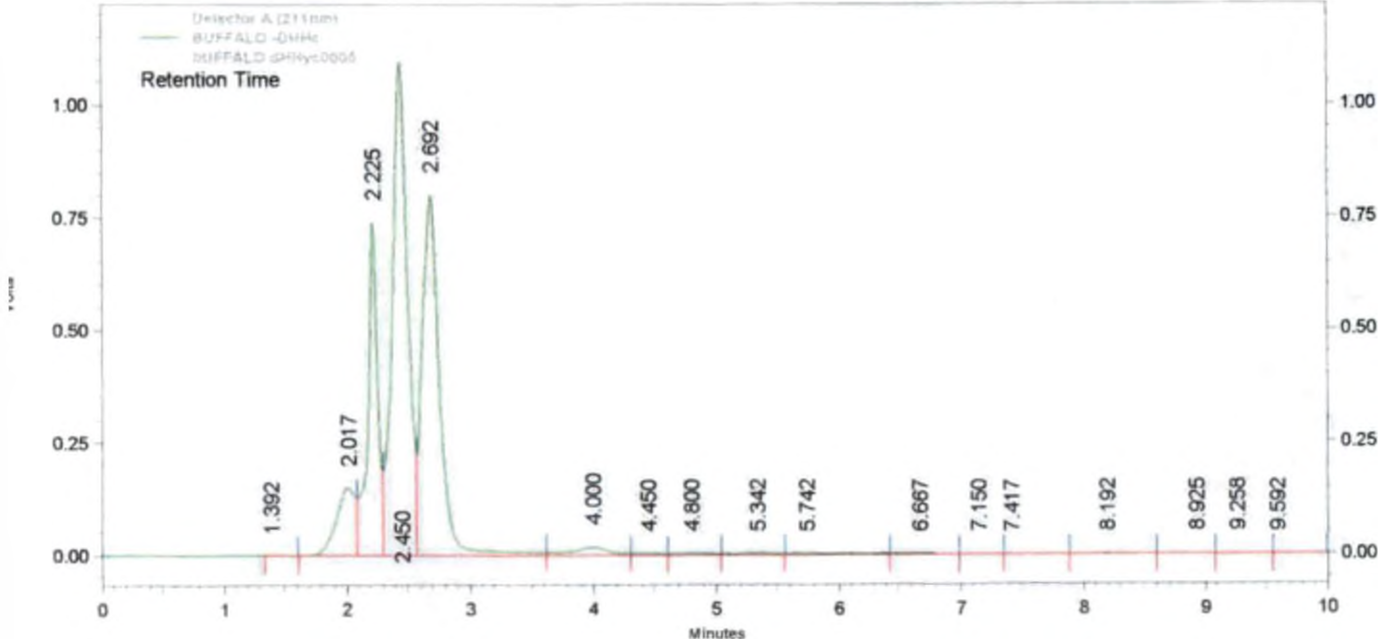


Figure.15 Chromatogram of buffalo fat used for carbofuran fortification and recovery study

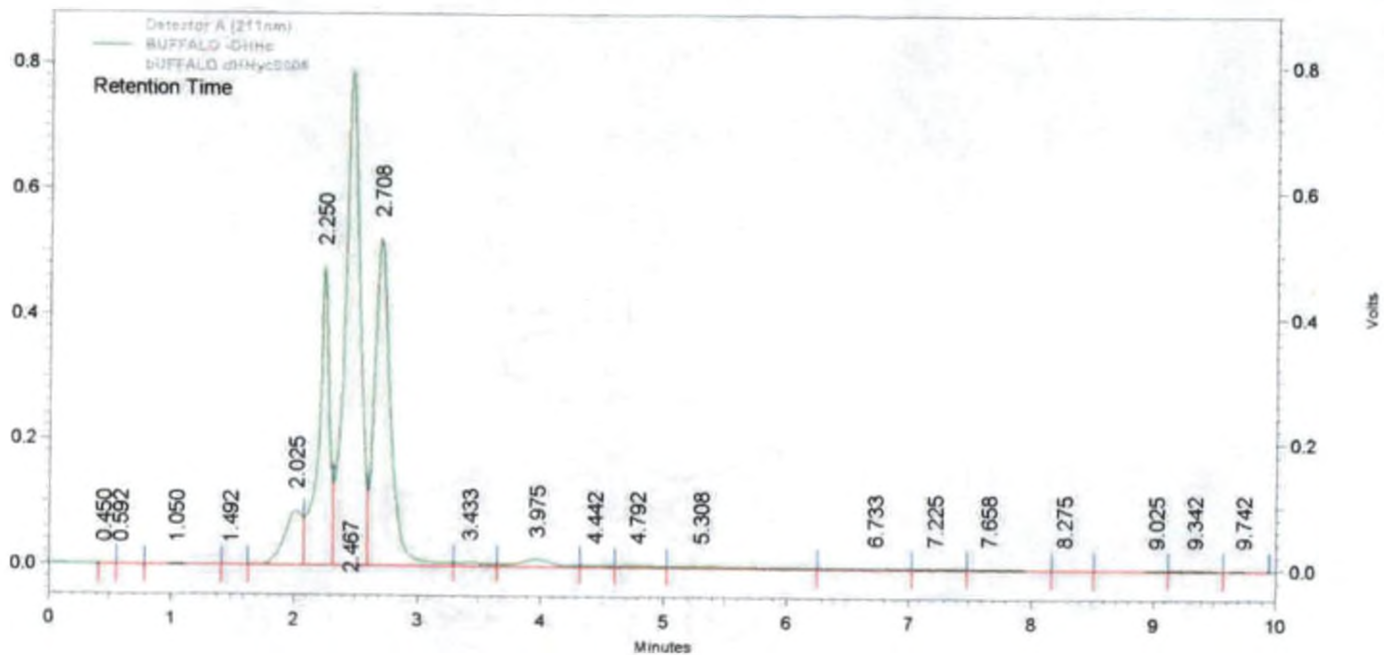


Figure.16 Chromatogram of buffalo meat screened for the residues of carbofuran and its metabolites

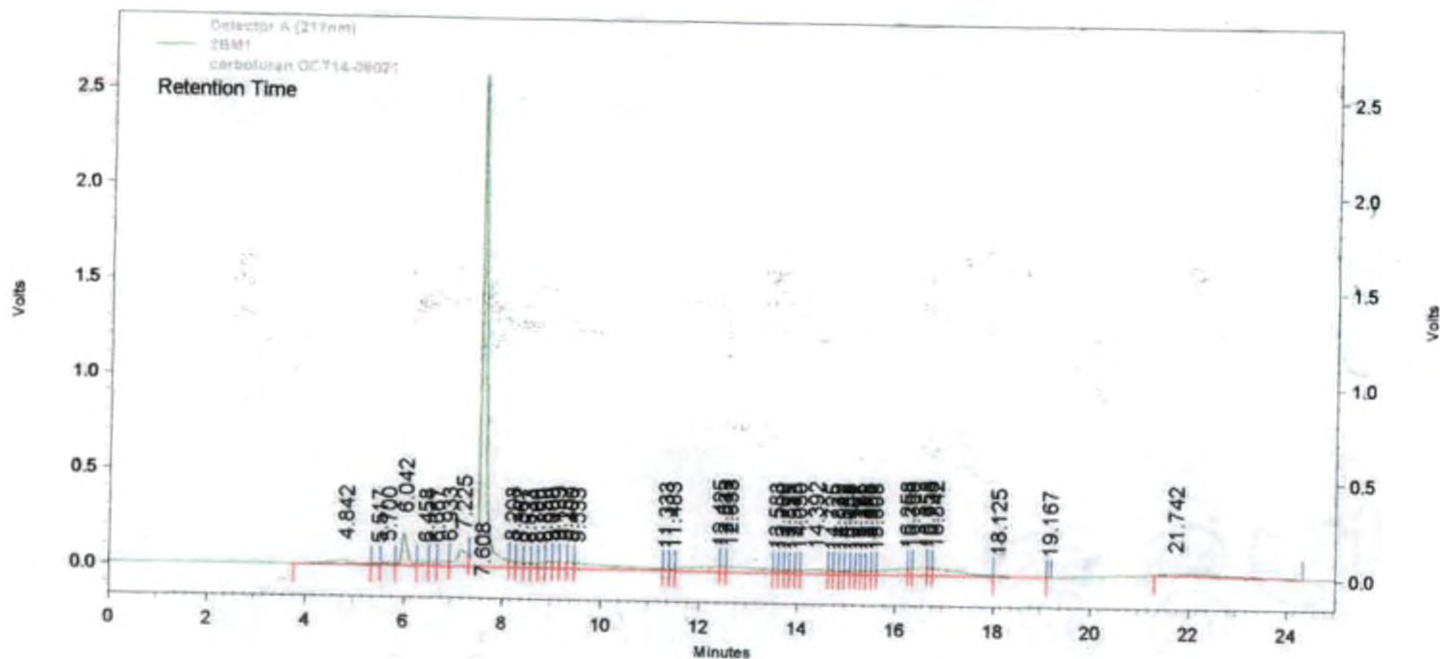


Figure.17 Chromatogram of buffalo liver screened for the residues of carbofuran and its metabolites

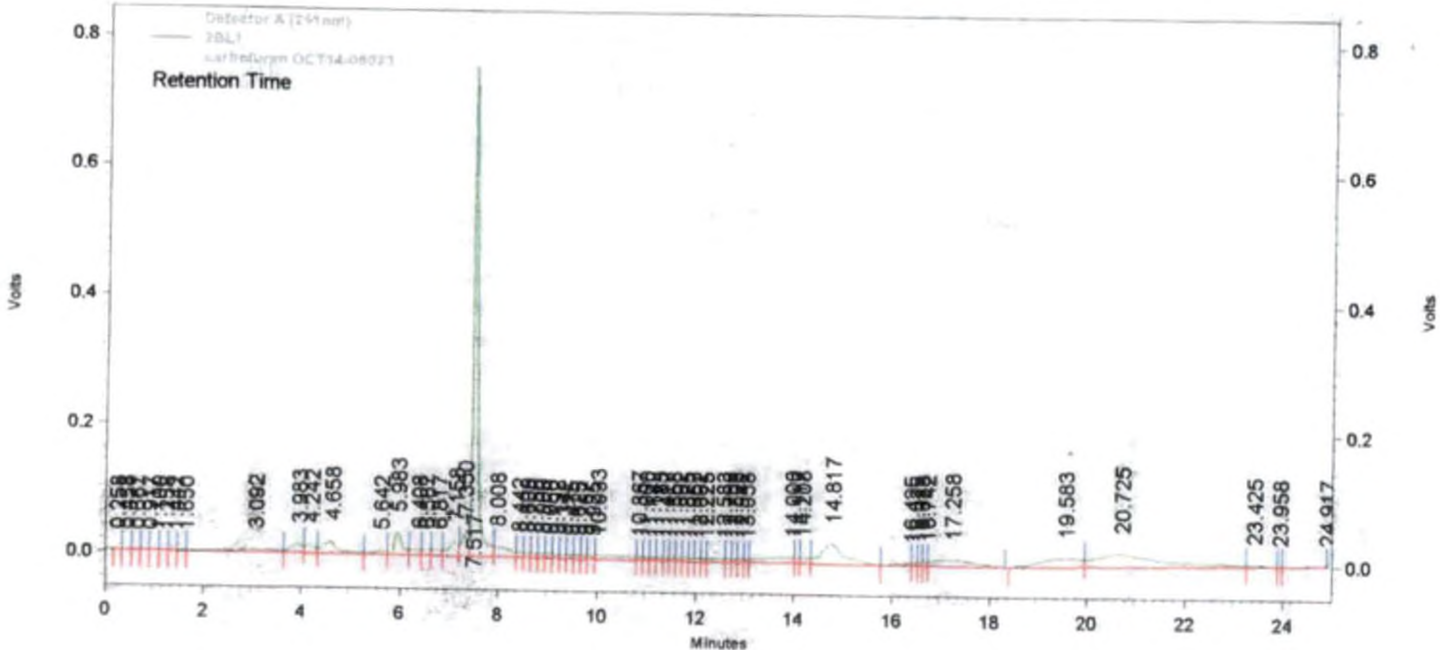


Figure.18 Chromatogram of buffalo kidney screened for the residues of carbofuran and its metabolites

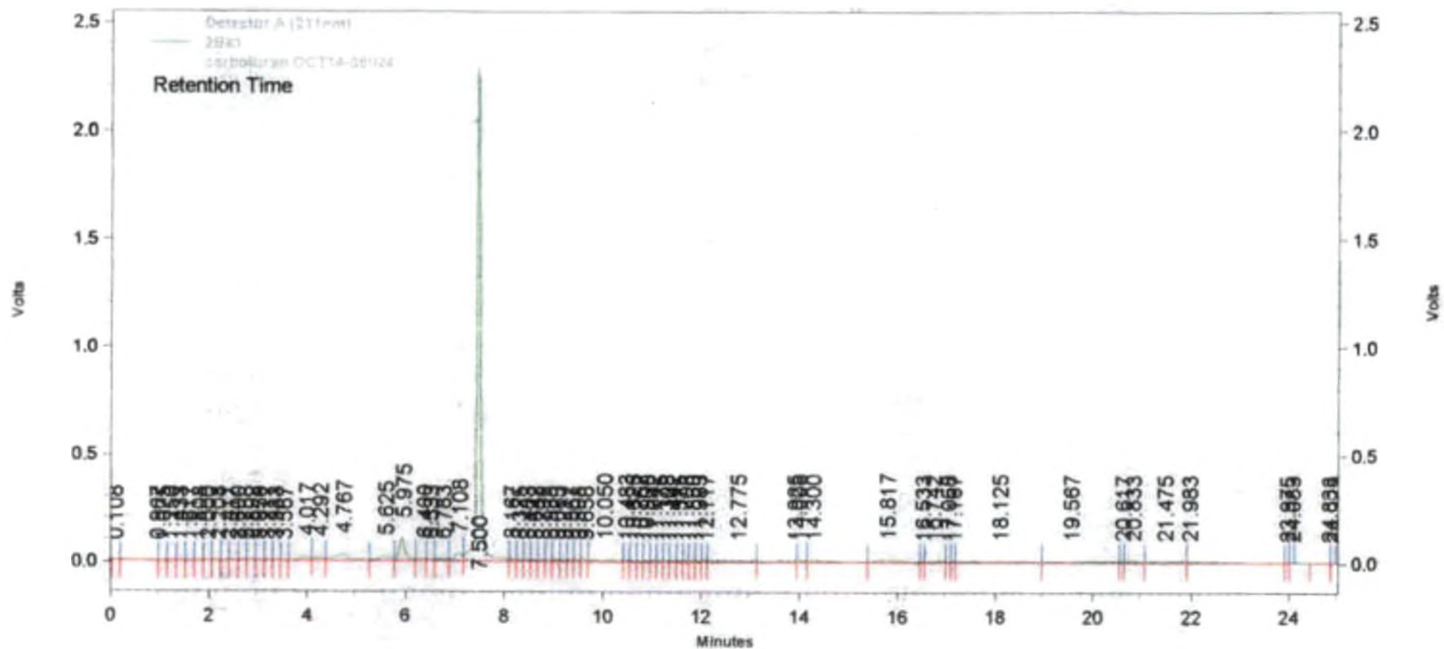


Figure.19 Chromatogram of buffalo fat screened for the residues of carbofuran and its metabolites

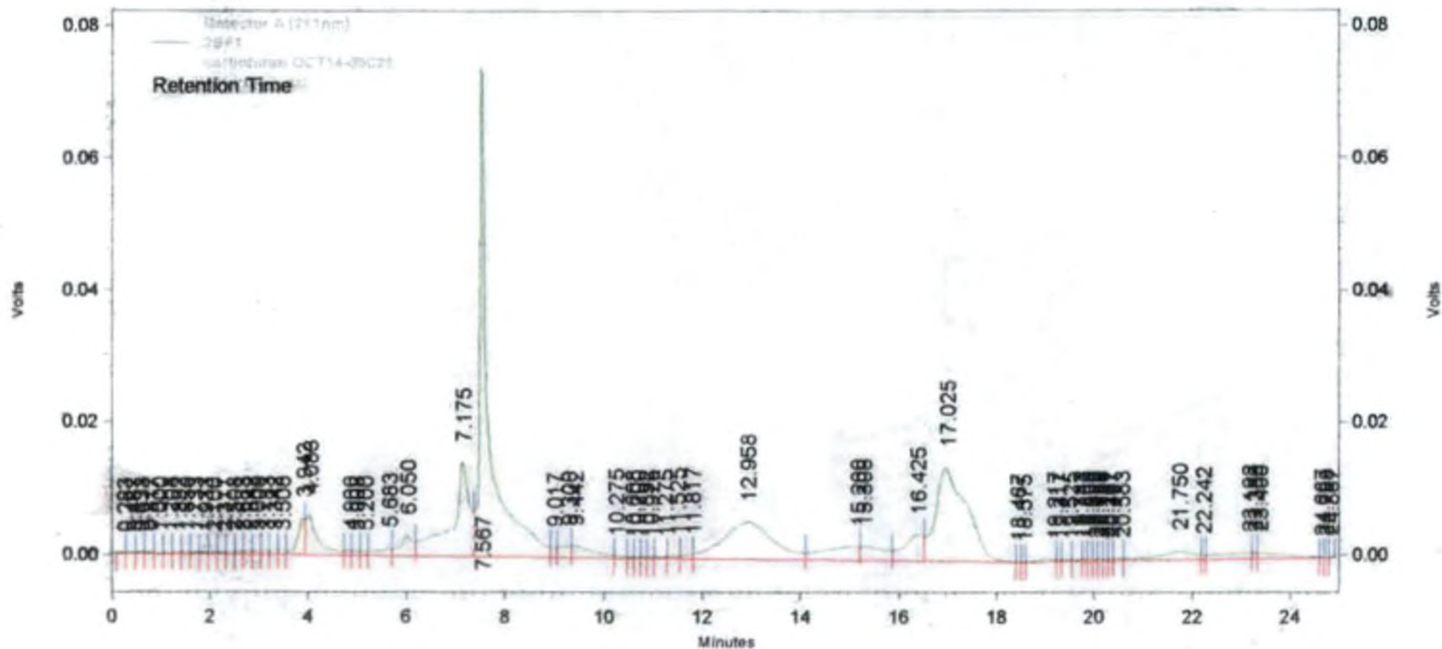


Figure.20 Chromatogram of duck meat screened for the residues of carbofuran and its metabolites

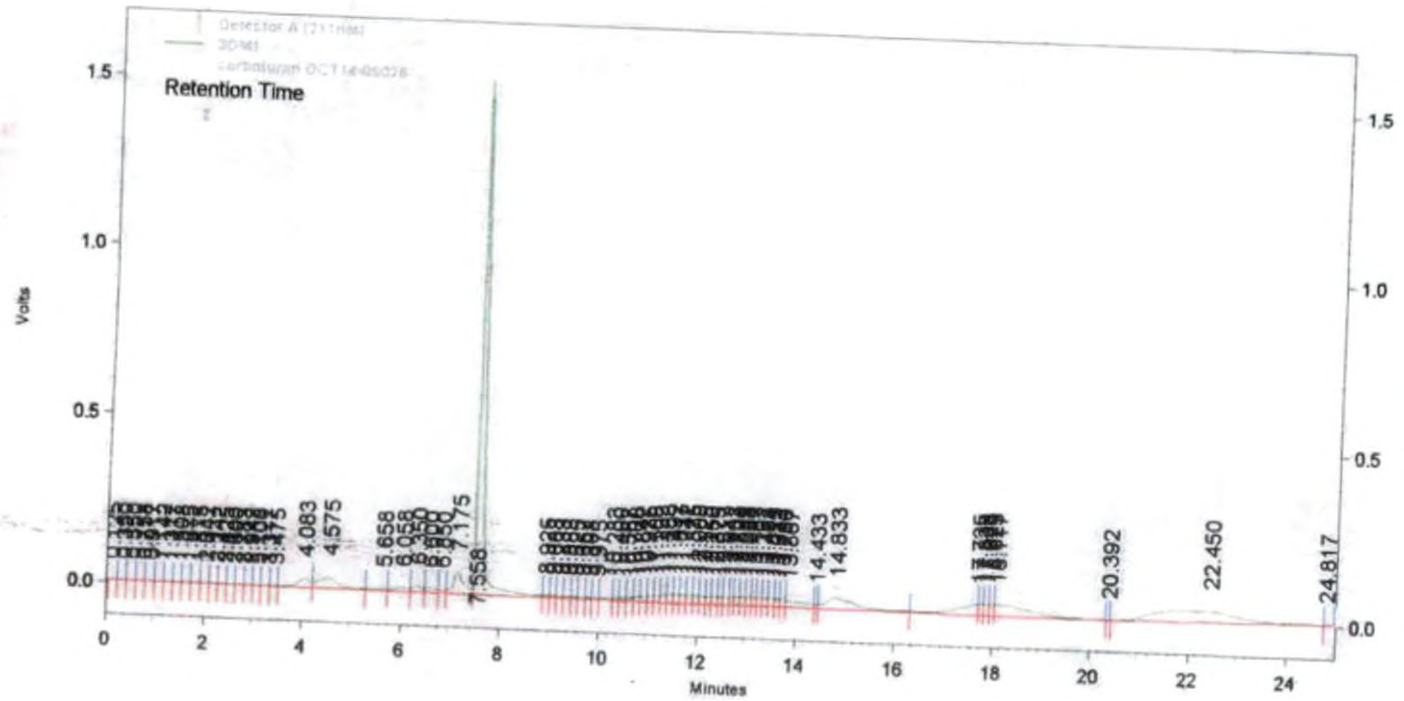


Figure.21 Chromatogram of duck liver screened for the residues of carbofuran and its metabolites

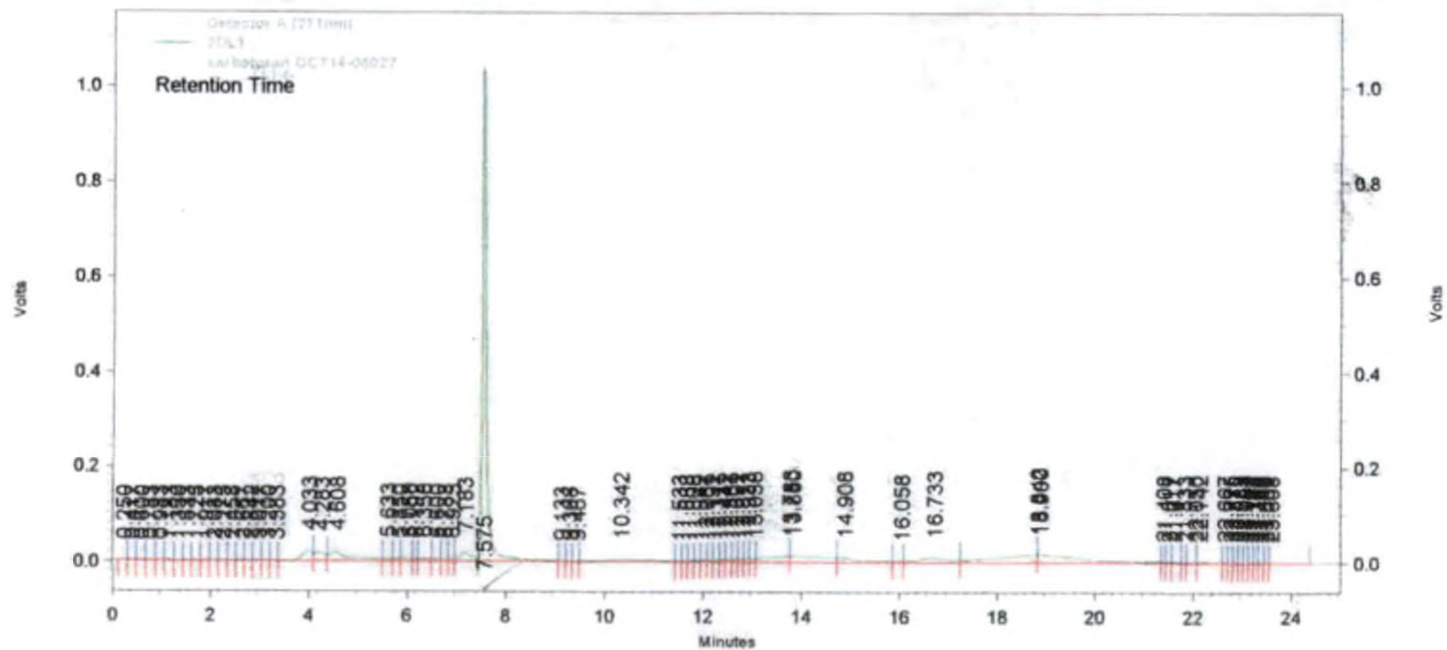


Figure.22 Chromatogram of duck kidney screened for the residues of carbofuran and its metabolites

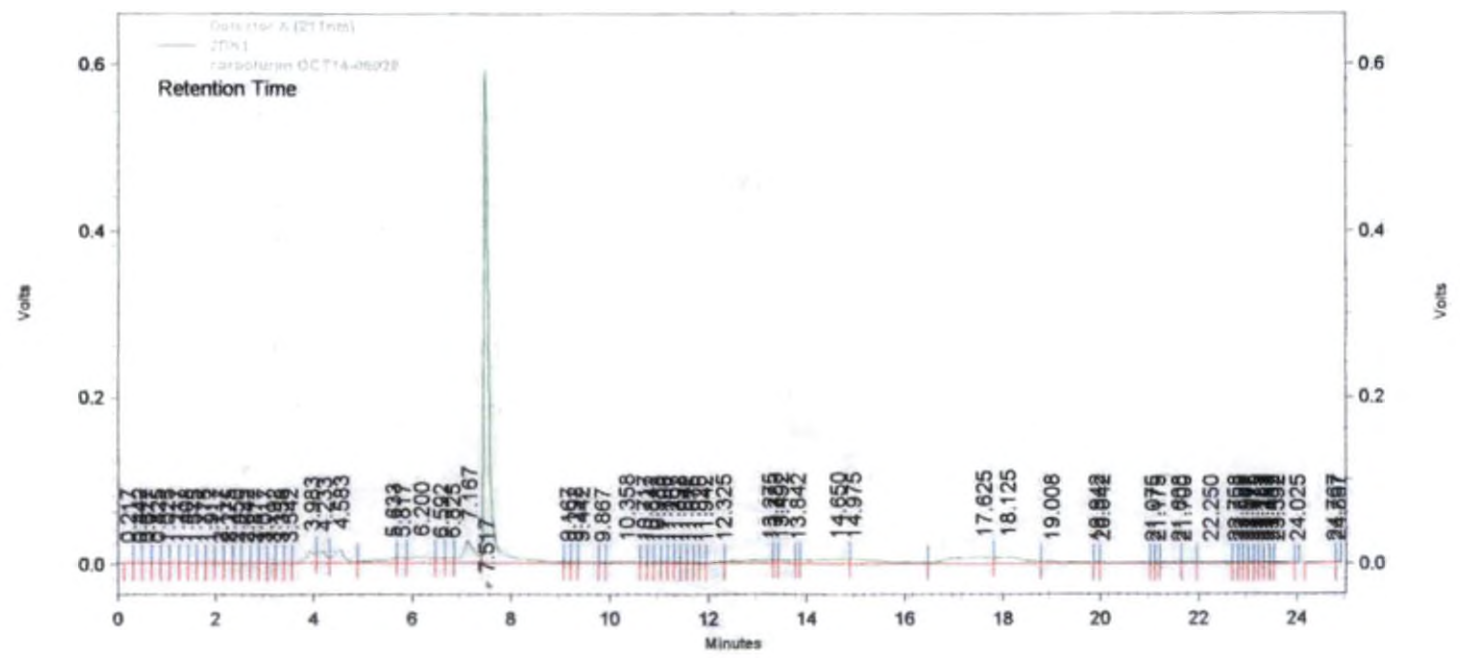
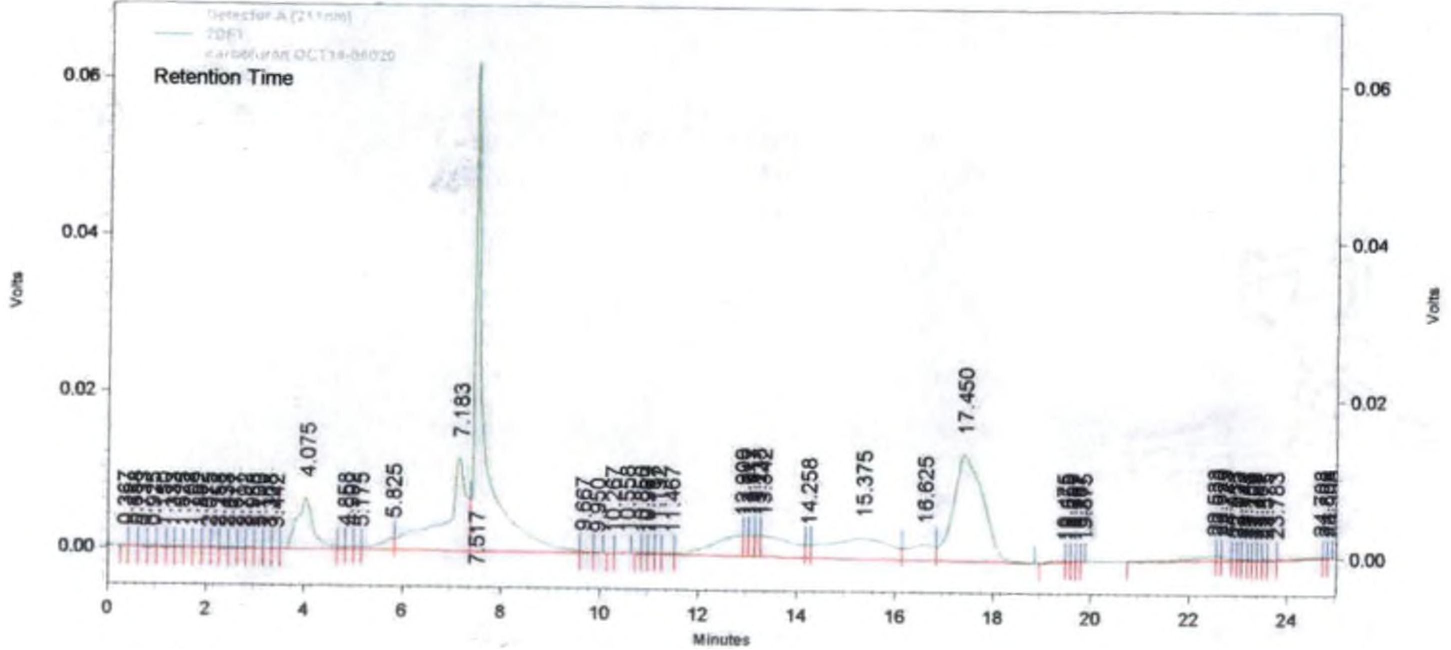


Figure.23 Chromatogram of duck fat screened for the residues of carbofuran and its metabolites



Discussion

172568

DISCUSSION

A research work on determination of residues of carbofuran and its metabolites in tissues of buffaloes and ducks was undertaken in the present study. The objectives were to develop and standardise a suitable simple method of extraction of residues of carbofuran and its metabolites from meat, liver, kidney and fat of buffaloes and ducks for reversed phase HPLC analysis and to apply the method in the screening of animal tissues for these residues.

5.1 HPLC OPERATIONAL CONDITIONS

Carbofuran is a broad spectrum noncumulative systemic carbamate insecticide which is soluble in water and organic solvents like ACN, hexane, acetone, methanol and 2-propanol. HPLC method is found to be the most suitable method for residue screening for carbamate compounds (Aaron and Coly, 2000) and so HPLC was resorted to for the determination of carbofuran and its metabolites.

In the HPLC analysis of carbofuran and its metabolites, a commercial reversed phase C 18 column bonded to silica was used as suggested by Soriano *et al.* (1998). This column was regenerated with pure polar solvent diluted with water. In reversed phase HPLC, such a combination would enable in cleaning of the pumps and columns, separation of pesticides and regeneration of the columns.

The operational parameters such as mobile phase, composition, flow rate, and detection wave length for the analysis were optimised by trial and error method.

In the study when ACN alone was used as the mobile phase at a flow rate of 1 ml/min. the compound 3-hydroxy carbofuran did not elute within 20 min. After trying with different ratios of ACN/water, a final ratio of 35:65 was fixed where an elution time of 5.542 min. was obtained at a flow rate of 1 ml/min.

Similarly, for carbofuran an elution time of 17.258 min. was obtained at the same mobile phase and flow rate. When water is added to ACN, the polarity of the solvent increases, whereby the efficiency of separation of such compounds increases in reverse phase columns. The combination of ACN and water as the mobile phase was reported by (Krause, 1988) in the analysis of carbamate pesticide residue by HPLC.

For a method to be efficient, it is inevitable that the parent compound as well as any of its metabolites could be analysed within a minimum run time. So, the parent compound carbofuran and its metabolite 3-hydroxy carbofuran were focused in optimising the flow rate. In order to reduce the retention time, the flow rate for carbofuran was increased to 1.3 ml/min. in the mobile phase of ACN/water 35:65. and an elution time of 14.058 min was obtained. Accordingly, the flow rate was time programmed (0.7 ml/min to 1.3 ml/min) and separation of carbofuran and its metabolites was possible within 25min. run time.

In the present study the UV detector wavelength was set to 211nm after confirming the absorbance by variable wavelength UV/VIS detector of the system. The pesticide carbamate has an aromatic moiety and UV absorbing group and therefore fixed/ variable wavelength UV detector for HPLC is applicable (Zweig, 1984). Carbamate compounds show better UV absorbance at 210 nm (Soriano *et al.*, 1998).

5.2 STANDARDISATION

5.2.1 Linearity Study

The linearity of the certified standards of carbofuran and 3-hydroxy carbofuran was checked at different concentrations of 1, 10 and 100 ppm in ACN An excellent linearity was obtained which is supportive for standardisation of the method.

5.2.2 Recovery Study

Different solvents, viz., acetonitrile, acetone, hexane, methanol and 2-propanol were employed for the extraction of residues of carbofuran from tissues of buffalo such as meat, liver, kidney and fat. Initially, carbofuran was spiked at 100 ppm level in different matrices and extracted with different solvents. Based on the percentage of recovery, acetonitrile was chosen as solvent for extraction of residue from meat, liver and kidney while hexane was the best solvent for extraction of residue from fat. A high recovery rate was observed which added to the reliability of the method.

This solvent system was applied in the recovery study of carbofuran from buffalo tissues at different fortification levels to assess its suitability for recovery of lower concentrations. It was found that the recovery rates ranged from 82-90 per cent. This is in agreement with the reports of Ali (1989) who obtained recovery rates > 80 per cent on fortification of beef and duck liver sample with mixed carbamate pesticide standards.

5.3 SAMPLE SCREENING

Tissue samples of buffaloes and ducks were collected at random from different parts of Kerala. The analytical method developed was employed in screening these samples for residues of carbofuran and its metabolites, viz., 3-hydroxy carbofuran, 3-keto carbofuran, 3-hydroxy 7-phenol carbofuran, 3-keto 7-phenol carbofuran and carbofuran phenol. In buffalo meat, liver, kidney and fat the residues could not be detected and therefore, designated as below detection limit (BDL). In duck also similar results were obtained except in one sample in which 3-hydroxycarbofuran residue was detected in meat and kidney at the level of 2 ppm and 0.9 ppm, respectively. This level is above the MRL of 0.1 ppm. The residue is defined for compliance with MRLs as the sum of carbofuran and 3-hydroxycarbofuran. These ducks might have been exposed to carbofuran through water, feed, fish or other invertebrates which they consumed from the

rice fields. Kwon *et al.* (2004) attributed carbofuran poisoning as the cause of mortality in mallard and mandrin ducks. Elliot *et al.* (1996) reported that granular carbofuran persisted long enough in the wet low pH conditions in the soil for several months after its application.

The results of the present study reveal that residues of carbofuran and its metabolites are not found in detectable levels in the samples of animal tissue. This may be due to the non cumulative action of the compound. However, monitoring of these residues is essential because of greater usage in agriculture. So an appropriate method is developed for the screening of animal tissues to detect the presence of these residues. This method is simple without much purification techniques like Solid Phase Extraction and with a detection limit of 0.1 ppm. This would enable in monitoring and surveillance of residues of carbofuran in animal tissues, hazard analysis and determining the critical control points.

Summary

SUMMARY

A research work on determination of residues of carbofuran and its metabolites in tissues of buffaloes (*Bubalus bubalis*) and ducks (*Anas platyrhynchos*) was under taken in the present study. HPLC analysis is found to be the most suitable method for residue screening for carbamates and only a few methods are developed for extraction of carbofuran residues from animal tissues. Therefore, the objectives of the present study were to develop and standardise a suitable simple method of extraction of residues of carbofuran and its metabolites from meat, liver, kidney and fat of buffaloes and ducks for reversed phase HPLC analysis and to apply this method in the further screening of animal tissues for monitoring these residues.

Fifteen samples of each tissue of buffaloes and ducks, viz., muscle, liver, kidney and fat were randomly collected from different lots of slaughtered animals in Kerala according to the Guidelines of Codex Alimentarius Commission. Different solvents, viz., acetonitrile, acetone, methanol, hexane and 2-propanol were used for the extraction of carbofuran residues and its metabolite from tissues. The extracted samples were cleaned up by liquid-liquid partitioning and purified by filtration through disposable cartridges with 0.2 μ cellulose filters.

The HPLC system used was Schimadzu LC- 10 AVP series with manual injection, reversed phase C 18 (2) 5 μ column and variable wavelength UV/VIS detector installed with Class VP Software. Operational parameters were standardised as: mobile phase- ACN/water 35: 65, sample volume 20 μl, flow rate: 0.7-1.3 ml/min. and detector wavelength: 211nm.

Certified standard references of carbofuran and its metabolites, viz., 3-hydroxy carbofuran, 3-keto carbofuran, 3-hydroxy 7-phenol carbofuran, 3-keto 7-phenol carbofuran and carbofuran phenol were dissolved in ACN at different concentrations. The samples of above standards were analysed in HPLC to identify the chromatogram peaks of respective compounds.

The linearity of the reference standard compounds carbofuran and its metabolite 3-hydroxy carbofuran was assessed by plotting a graph of the 1, 10 and 100 ppm concentration against area under the respective chromatogram peaks. Excellent linearity was observed for these standards.

In order to assess the percentage recovery of carbofuran by different solvents, buffalo tissues, viz., meat, liver, kidney and fat were spiked with 100 ppm carbofuran standard. Then the residues were extracted with different solvents like acetonitrile, acetone, methanol, 2-propanol and hexane for clean up and further analysis in HPLC.

The recovery of carbofuran from buffalo meat using acetonitrile was in the range of 89 to 95, 87 to 91, 89 to 91 and 56 to 58 per cent, respectively for meat, liver, kidney and fat. The recovery of acetone extracted carbofuran from meat, liver, kidney and fat was 39 to 42, 45 to 48, 41 to 43 and 36 to 40 per cent, respectively. When methanol was used for extraction, the respective recovery of carbofuran from meat, liver, kidney and fat was 2 to 5, 10 to 12, 7 to 9 and 14 to 16 per cent. For the extraction method using 2-propanol, the recovery of carbofuran was 6 to 8, 4 to 10, 9 to 11 and 13 to 15 per cent, respectively from meat, liver, kidney and fat. The recovery of hexane extracted carbofuran from meat, liver, kidney and fat was 50 to 56, 46 to 51, 49 to 53 and 86 to 93 per cent, respectively. Based on the highest percentage of recovery, ACN was selected as the best solvent for extraction of residues from meat, liver and kidney and hexane for fat.

This solvent system was applied for the extraction of residues in a further HPLC residue monitoring programme in the tissues of buffaloes and ducks. In buffalo meat, liver, kidney and fat the residues of carbofuran and its metabolites could not be detected and hence denoted as below detection limit (BDL). Residues could not be detected in all duck tissue samples, except in one sample,

wherein 3-hydroxy carbofuran was detected at 2 ppm and 0.9 ppm in meat and kidney, respectively which is above the MRL.

An appropriate method is developed for the screening of animal tissues to detect the presence of these residues. This method is simple without much purification steps like Solid Phase Extraction, and with a detection limit of 0.1 ppm. This would enable in monitoring and surveillance of residues of carbofuran in animal tissues, hazard analysis and determining the critical control points.

References

REFERENCES

Aaron, J.J and Coly, A. 2000. Luminescence methods in pesticide analysis: Applications to the environment. *Analusis*. 28: 8

Ahdaya, S and Guthrie, F.E. 1982. Stomach absorption of intubated insecticides in fasted mice. *Toxicol.* 22: 311-317

Ali, M.S.1989. Determination of N-methyl carbamate pesticides in liver by liquid chromatography. *J.Assoc.Off.Anal.Chem.Int.*72 (4): 586-92

Bakowski, R.S., Ali, M.S., White, J.D., Phillipo, E.T and Ellis, R.L. 1994. Gas chromatographic/Mass spectrometric confirmation of ten N-methyl carbamate insecticides in liver. *J.Assoc.Off.Anal.Chem.Int.*77 (6): 1568-74

Baligar, P.N and Kaliwal, B.B. 2003. Temporal effect of cabofuran, a carbamate insecticide in the interruption of the estrous cycle and follicular toxicity in female Swiss albino mice.*Bull.Environ.Contam.Toxicol.*71: 2,422-428

Bhatkar, N.V and Dhande, R.R. 2000. Furudan induced hematological changes in the fresh water fish, *Labeo rohita*. *J.Ecotoxicol.Env.Monitoring.*10: 3-4,193-196

*Bloch.1987. 13 week oral toxicity (feeding) study with carbofuran in the dog. Unpublished report submitted to WHO by FMC Corp. Cited in report of 1997 meeting of the core assessment group, FAO/WHO Joint Meeting on Pesticides.

CAC.1999. Report of the thirtieth session of the Codex Committee on Pesticide Residues, 1998. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome.

Chakravarthy, A., Pal, S., Kole, R.K., Banerjee, H and Bhattacharya, A. 2000. Supervised field trials of carbofuran and endosulfan in maize. *J.Interaccademicia.* 4: 3, 414-419

*Chen, A.W. 1995. Residue analytical method for the determination of carbosulfan and its major metabolites in cow meat, meat by-product and milk. Unpublished FMC Report.No.P-3065 M.cited in FAO 2004.

Draper, W.M., Gibson, R.D and Street, J.C. 1981. Drift from and transport subsequent to a commercial, aerial application of carbofuran: an estimation of potential human exposure. *Bull. Environ. Contam. Toxicol.* 26: 537-543

Elliot, J.E., Langlier, K.M., Mineau, P and Wilson, L.K. 1996. Poisoning of bald eagles and red tailed hawks by carbofuran and fensulfothion in the Fraser Delta of British Columbia, Canada. *J.Wildlife Diseases.* 32(3): 486-491

FAO. 2004. Plant Production and Protection Paper 176.*Pesticide Residues in Food Report 2003*-Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group, Food and Agriculture Organization, Rome.

FAO/WHO. 1977. *Evaluations of some pesticide residues in food-1976.* Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group, Food and Agriculture Organization, Rome.

FAO/WHO.1985. Data Sheet on Pesticides No.56 (VBC/PDS/DS 85-56), Food and Agriculture Organization, Rome.

FAO/WHO. 1997. Pesticide residues in food-1996. Joint FAO/WHO Meeting on Pesticide Residues. Evaluations 1996.Part II-Toxicologocal.International Programme on Chemical Safety, World Health Organisation, Geneva.

FAOSTAT. 2006. http:// faostat.fao.org.

Ganguly, N.K., Medappa, N and Srivastava, V.K. 2001. Pesticide pollution: Trends and perspectives. ICMR Bulletin. 31: 9

Garcia deUasera, M.P and Bernal, G.M. 2001. Presence of carbamate pesticides in environmental waters from the northwest of Mexico: determination by liquid chromatography. *Water R es.* 35(8): 1933-40

*Ginzburg, N. 2001. Final Report-Determination of residues of carbosulfan and its metabolites and 3-hydroxy carbofuran in meat, milk and eggs-validation of the method .FMC unpublished report, FA-17-00-29.cited in FAO 2004.

*Goldenthal, E.I. 1982. 2-year dietary toxicity and carcinogenicity study in rats. Unpublished report submitted to WHO by FMC Corp. Cited in report of 1997 meeting of the core assessment group, FAO/WHO Joint Meeting on Pesticides

Goswami, M.M., Deka, K.C., Barooah, A.K and Borah, B. 2000. Residues of carbofuran and endosulfan in maize.*J.Agric.Sci.Society of NorthEast. India.*13: 1, 12-15

Gunathilagaraj, K and Ramesh, P.T. 2000. Toxicity of endosulfan and carbofuran to earthworms. *Madras Agric. J.* 87: 4-6,323-324

Gunier, R.B., Karnly, M.E., Reynolds, P., Hertz, A and Behren, J.V. 2001. Agricultural pesticide use in California: Pesticide prioritization, due densities and population distribution for a childhood cancer study. *Env.Health Perspect.* 109 (10): 1071-1077

Health and Welfare Canada. 1991. Guidelines for Canadian drinking water quality. Ottawa, Ontario.

Hill, E.F and Fleming, W.J. 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Env.Toxicol.Chem.*1: 27-38

Holstege, D.M., Scharberg, D.L., Tor, E.R., Hart, L.C and Galey, F.D. 1994. A rapid multiresidue screen for organophosphorus, organochlorine and

N-methyl carbamate insecticides in plant and animal tissues. *J.Assoc.Off.Anal.Chem.Int.*77 (5): 1263-74

Hunt, K.A., Hooper, M.J and Littrell, E.E. 1995. Carbofuran poisoning in herons: diagnosis using cholinesterase reactivation techniques. *J.Wildlife Diseases*.31 (2): 186-192

Indiradevi, P. 2006. Pesticide use in rice production and human health-A study in Kerala, Kerala Agricultural University and South Asian Network for Development and Environmental Economics. Policy Brief Bulletin, March 2006.

IPCS. 1997. Report of the 1997 meeting of the Core Assessment Group, Joint Meeting on Pesticides. International Programme of Chemical Safety, WHO, Geneva.

Jeon, S.D., Lim, J.S and Moon, C.K. 2001. Carbofuran suppresses T-cell mediated immune responses by the suppression of T-cell responsiveness, the differential inhibition of cytokine production, and NO production in the macrophages. *Toxicol.Letters*.119; 2,143-155

Kannathasan, M., Krishnaveni, S.A and Padmaja, B. 2001. Leaching and movement of carbofuran and butachlor in major soil series of rice tract of Madurai district. *Madras Agric. J.* 88: 4-6, 348-350

Kawamoto, T and Makihata, N. 2003. Development of a simultaneous analysis method for carbofuran and its three derivative pesticides in water by GC/MS with temperature programmable inlet on column injection. *Anal. Sci.*19: 1605-1610

Khatoon, J.A and Chowdhury, M.A.Z. 2001. Bioavailability and toxicological potential of 14C carbofuran residues in rat. *Bangladesh Veterinarian*.18: 1, 70-75

Kimbrough, R.A and Litke, D.W. 1996. Pesticides in streams draining agricultural and urban areas in Colorado. *Environ.Sci.Toxicol.* 30:908-916

Krause, R.T. 1988. High performance liquid chromatographic determination of aryl N-methyl carbamate residues using pest-column hydrolysis electrochemical detection. *J. Chromatogr.* 442: 333-43

Kwon, Y.K., Wee, S.H and Kim, J.H. 2004. Pesticide poisoning events in wild birds in Korea from 1998 to 2002. *J.Wildlife Diseases.* 40(4): 737-740

Laveglia, J. 1981. Teratology study in the rabbit with carbofuran Unpublished report submitted to WHO by FMC Corp. Cited in report of 1997 meeting of the core assessment group, FAO/WHO Joint Meeting on Pesticides.

*Littrell, E.E.1988. Waterfowl mortality in rice fields treated with carbamate, carbofuran. *California Fish and Game*.74: 226-231

*Liu, K.H., Byoun, J.Y., Sung, H.J., Lee, H.K., Kim, K., Lee, H.S and Kim, J.H. 2001. Simultaneous determination of furathiocarb and metabolites in biological tissues by high performance liquid chromatography and post column derivatization. *Chromatographia*.53: 11-12

Mathur, S.C. 1999. Future of Indian pesticide industry in next millennium. *Pestic.Inf.* 24 (4): 9

McMahon, B.M and Wagner, R.F. 1994. *Pesticide Analytical Manual, Volume 1: Multiresidue methods.* Third edition. Food and Drug Administration, U.S.A

MFPO.1973. Meat and Food Products Order 1973, Ministry of Agriculture,Govt. of India..

PFA. 1997. *Prevention of Food Adulteration Act, 1954.* Nineteenth edition. Eastern Book Company, Lucknow. p.104.

Quick, M.P. 1982. Pesticide poisoning of livestock: A review of cases investigated. *The Vet. Rec.* 111: 5-7

Ram, R.N., Singh, I.J and Singh, D.V. 2001. Carbofuran induced impairment in the in the hypothalamo-nuerophyseal-gonadal complex in the teleost, *Channa punctatus* (Bloch). *J. Environ. Biol.* 22: 3,193-200

Reddy, K.N., Rao, B.N., Sulthan, M.A and Reddy, D.J. 2000. Monitoring of pesticides in total diet. *J.Res.ANGRAU.* 28: 4, 100-103

Sivakumar, T and Jiji, T. 2003. Preference of chemicals among snake gourd farmers in Kerala, South India. *Insect-Environment.*9: 1, 37-38.

Snedecor, G.W and Cochran, W.G. 1994. *Statistical Methods.* Eighth edition.The Iowa State University, Ames, Iowa, USA.313 p.

Soriano, J.M., Jimanez, B., Redondo, M.J and Molto, J.C. 1998. Comparison of different sorbents for online liquid solid extraction. followed by high performance liquid chromatographic determination of nitrogen containing pesticides. *J. Chromatogr. A.*822 (1):67-73

Street, J.C and Sharma, R.P. 1995. Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern. Quantitative studies of immunosupression by DDT, Aroclos1254, carbaryl, carbofuran and methyl parathion. *Toxicol.Appl.Pharmacol.* 32: 587

Sudipta, C., Dasmahapatra, A.K., Rama, G., Chatterjee, S. and Gosh, R. 2001. Disruption of pituitary–ovarian axis by carbofuran in catfish, *Heteropneustes fossilis* (Bloch). *Comp.Biochem.Physiol.*129: 3,265-273

Szeto, S.Y and Price, P.M.1991. Persistence of pesticide residues and mineral and organic soils in the Fraser valley of British Columbia. *J. Agric. Food Chem.* 39: 1679-1684

Tripathi, G., Amit, S., Poonam, B., Sharma, A and Bharadwaj, P. 2002. Carbofuran induced modification in cellular activities and mineralization

172568

potential of an endemic earthworm, *Lampito mauritii*. *Biochemical and Cellular Archives*. 2: 1-2, 75-81

WHO.1996a. Guidelines for drinking water quality, Second edition.Vol.2.Health criteria and other supporting information, World Health Organisation, Geneva.

WHO.1996b. The WHO recommended classification of pesticides by hazards and guidelines to classification. World Health Organisation, Geneva. (WHO/PCS/96.3)

Williams, I.H., Brown, M.J and Whitehead, P.E.1976. Persistence of carbofuran residues in some British Columbia soils. *Bull. Environ. Contam. Toxicol.* 15: 242-243

*Wilson, L.K., Moul, I, E., Langlier, K.M and Elliot, J.E. 1995. Summary of bird mortality in British Columbia and Yukon, 1963-1994.Technical Report Series No.227, Canadian Wildlife Service, Delta, British Columbia, Canada.214p.

Yang, S.S., Goldsmith, A.I and Smetena, I. 1996. Recent advances in the residue analysis of N-methyl carbamate pesticides. *J. Chromatogr. A*. 754: 3-16

Yoona, J.Y, Oha, S.H., Yoona, S.M., Leea, S.J., Leea, H.S., Choia, S.J., Moon, C.K and Lee, B.H. 2001. N-Nitrosocarbofuran, but not carbofuran induces apoptosis and cell cycle arrest in CHL cells. *Toxicology*.169: 2,153-161

Zweig, G. 1984. Recent developments in the analysis of pesticides. *Modern methods of food analysis*. (Eds. Stewart, K.K. and Whitaker, J.R.). AVI Publishing Company, Inc.Connecticut, pp.339-368

*Originals not consulted

DETERMINATION OF RESIDUES OF CARBOFURAN AND ITS METABOLITES IN TISSUES OF BUFFALOES AND DUCKS

POULSON JOSEPH

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary & Animal Sciences
Kerala Agricultural University
Thrissur

2006

Department of Livestock Products Technology
COLLEGE OF VETERINARY & ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

A research work on the determination of residues of carbofuran and its metabolites in tissues of buffaloes (*Bubalus bubalis*) and ducks (*Anas platyrhynchos*) was under taken. The objectives were to develop and standardise a suitable simple method of extraction of residues of carbofuran and its metabolite from meat, liver, kidney and fat of buffaloes and ducks for reversed phase HPLC analysis and to apply this method for further screening of animal tissues for monitoring these residues

Fifteen samples of each tissue of buffaloes and ducks were randomly collected from different lots of slaughtered animals in Kerala. Different solvents, viz., acetonitrile (ACN), acetone, methanol, hexane and 2-propanol were used for the extraction of carbofuran residues and its metabolite from tissues. The extracted samples were cleaned up by liquid-liquid partitioning for HPLC analysis using Schimadzu LC-10 AVP series with UV/VIS detector at 211 nm in a mobile phase of acetonitrile (35): water (65).

Certified standard references of carbofuran and its metabolites, viz., 3-hydroxy carbofuran, 3-keto carbofuran, 3-hydroxy 7-phenol carbofuran, 3-keto 7-phenol carbofuran and carbofuran phenol were analysed in HPLC to identify the chromatogram peaks of respective compounds. Excellent linearity was observed for carbofuran and 3-hydroxy carbofuran at 1, 10 and 100 ppm. Based on the highest percentage of recovery, ACN was chosen as the best solvent for extraction of residues from meat, liver and kidney and hexane for fat.

This solvent system was applied for the extraction of residues in a further HPLC residue monitoring programme in the tissues of buffaloes and ducks. In buffalo meat, liver, kidney and fat the residues of carbofuran and its metabolites could not be detected and hence denoted as below detection limit (BDL). Residues could not be detected in all duck tissue samples, except in one sample,

wherein 3-hydroxy carbofuran was detected at 2 ppm and 0.9 ppm in meat and kidney, respectively which is above the MRL. An appropriate method is developed for the screening of animal tissues to detect the presence of residues of carbofuran and its metabolites. This would enable in monitoring and surveillance of residues of carbofuran in animal tissues, hazard analysis and determining the critical control points.