



Forensic analysis of traces of bromadiolone rodenticide from soil at site where biological flesh sample buried

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Abstract : Bromadiolone is second generation single dose anticoagulant rodenticide like zinc phosphide. It is widely used to eradicate rodent from affected places and easily available in the form of the rat kill cake of various manufacturer i.e. Mortein , Tiger Mortein, Ratol etc. in Indian market. As a effective rodenticide, it is increasingly being used by people for committing suicide and even homicide. Authors conducted this experiment for the study of traces of bromadiolone in soil samples after the complete decomposition of the soft tissue where buried for forensic purpose. In this paper authors selected a Mortein rat kill bait of 25gm(0.005% of bromadiolone) five in numbers. A total 125gm of powdered rat kill cake subjected to 0.125gm of goat flesh without bone at the room temperature for one hour. This soft tissue of the flesh placed on the plastic pot between the layers of soil (pH - 7.5) at the room temperature. At every seven days of completion (i.e. 7,14,21,28 days) of the same sample has been observed for the presence of the maggot, pupa, colour, smell and stage of decomposition. And after the 28 days since 21 November 2019, soil samples taken out from pot and analyzed by Thin layer chromatography. Traces of bromadiolone from soil samples confirmed by TLC. Blue and brown spot has been developed and their Rf×100 values measured as 90 & 45 respectively.

Key Word: Bromadiolone, Rodenticide, Rat poison, TLC, soil

INTRODUCTION Bromadiolone(B) {3-[3-(4-Bromadiolone) Phenyl] 3-hydroxy-1-Phenylpropyl]-2-hydroxychromen-4-one. Molecular formula is C₃₀H₂₃BrO₄, was first registered in the united states in 1980, it is easily available in the form of the rat kill cake of various manufacturer Mortein ,Tiger Mortein, Ratol etc. In the Indian market as effective rodenticide, it belongs to family of coumarin, second generation (SGARs) single dose anticoagulant rodenticide like Zinc Phosphide. It is widely used to eradicate rodents from affected places. Bromadiolone is relatively persistent in environment , it is stable but rapidly binds on the soil with very slow desorption and without leaching, some studies indicate that it may take longer to break down if stored underground or in soil by flesh(NPIC.U.S), it has low potential to move up into the air. Bromadiolone is slightly soluble in water and completely soluble in Dimethylformamide, it has high, acute oral toxicity LD₅₀ of 1-3 mg/kg for various species including rodents and acute values for cats less than 25 mg/kg body weight, The liver is the main organ of accumulation and storage in the body,(0.005%) Bromadiolone used rodenticide pest control to kill rodents available in market 25gm mortin power guard rat kill ,it is increasiment being used by people for committing suicide and even homicide.

2. Materials and Methods

(0.005%) Bromadiolone sample brand name is Mortin power guard ratkil were purchase from the market these are easily available in the form of solid cake each 25g pack in red plastic wrapping. The soil sample was taken from the place-BHAMU Department of sport ground Aurangabad, brown colour, in dry condition and Goat flesh without bone 0.125g was taken from Liberty Butcher shop gulmandi Aurangabad, and plastic pots.

Before analyzing them, the samples were finely minced and dissolved in five different chemicals, like acetone , normal hexane alcohol, ethyle acetate and dimethylformamide and kept overnight to facilitate proper extraction. A filtrate of this solution was then collected in a clean and sterilized porcelain basin and kept

undisturbed for a few hours after drying the filtrate at room temperature a few millilitres of acetone was added to the residue. We took plastic pot which was divided in three parts first added soil at the bottom in the pot then mortein ratkil (powdered by mortar and pestle)with goat flesh, it having bromadiolone put on soil at top to bottom and covered with soil at top and left for the 28 days,at the room temperature at seven day of completion(7,14,21,28 days) of the same sample has been observed for the presence of the maggote, pupa ,colour, smell and decomposition stages and after 28 days soil sample taken out from pot and analyzed. Suspected soil sample about 25g and neutral soil sample did same procedure for extraction which was used for extraction of standard bromadiolone(0.005%).This solution was then spotted on a TLC plate, three spot bromadiolone pure, suspected soil sample and pure soil sample.The developing chamber was properly saturated with the solvent system, and the chamber after making a solvent run of 10cm from the point of spotting. This developed plate was then dried at room temperature.The details of the experimental condition have been given in **Table.1**. Obeservations were made under sunlight, ultraviolet light, and iodine fuming. These same sample analyzed by pH test,FT-IR, and Uv-visible spectrophotometer

Results and Discussion

Sample of bromadiolone were subjected to TLC analysis for the separation of its constituents. Acetone was found to be the best solvent for the extraction of these samples.seven different solvent systems were tried for the separation of this sample **Table 2**. Out of these seven solvent systems,three produced noticeable and significant results.The best solvent system was found to be Hexane:methanol:ethyl acetate(18:3:9).The other two solvent systems tried included one that Methanol:acetone:benzene(3:9:8) extracted by acetone,hexane,DMF and another that consisted of Hexane:ethyl acetate:acetic acid(3:2:1).These were studied under iodine fumes.Two spots(blue and brown)were usually visible in sunlight,while Uv-results could not produce any notable observation.It can be deduced from **Table 3** that the solvent system-B,E and G was the best solvent system for the separation of this poison from soil.

Table 1 Experimental Conditions of the study



Fig.1.Experimental setup

Table 1 Observation of decomposition stages flesh having Bromadiolone

Days	Smell	Condition Of flesh	Magot formation	Pupa	Pupa converted in To fly
After 7	Present	Colour changed blackish	Yes	No	No
After 14	present	Flesh start decomposition fastly	Yes	Yes	No
After 21	less	85% dicomposed	Yes	Yes	Yes
After 28	No	Flesh decomposed	Yes	Yes	Yes



Fig 2 Decomposition stages flesh having Bromadiolone

Table 1 Experimental Conditions of the study

Solvent system	Room temp	Saturation time	Run time
A	26°C	25 min	47min
B	26°C	25min	45min
C	26°C	25min	50min
D	26°C	25min	47min
E	26°C	25min	45min
F/G	26°C	25min	50min

Table 2 List of Solvent System

Sr no	code	Solvent system	Ratio
1	A	Hexane:DMF:DCM	(12:2:6)
2	B	Hexane:Methanol: Ethyl acetate	(18:3:9)
3	C	Hexane: Benzene:Ethyl alcohol: acetic acid	(6:2:1:1)
4	D	Hexane:Ethyl acetate	(21:9)
5	E	Hexane: Ethyl acetate: Acetic acid	(3:2:1)
6	F	Hexane: Benzene:DCM: Ethanol	(6:2:1:1)
7	G	Methanol:Acetone: Benzene	(3:9:18)

TLC Results – hRf values

Sample	Observation in Sun light no spot	hRf	UV/Long and short wavelength	Iodine fuming No spot
Pure Bromadiolone	One(Blue)	93	Nil	3 (12,35,78)
Suspected soil	One (Blue)	93	Nil	3(12,35,78)
Pure soil	No	No	Nil	2(18,41)

Table.3 hRf value solvent system B extracted from Hexane

sample	Observation in Sun ligh no spot	hRf	UV/Long and short wavelength	Iodine fuming No spot
Pure Bromadiolone	One (Blue)	98	Nil	2(278,67)
Suspected soil	One(Blue)	98	Nil	2(78,67)
Pure soil	No	No	Nil	1(1)

Table.4 hRf value solvent system B extracted in Acetone

Smple	Observation in sun light no spot	hRf	UV/Long and short wavelength	Iodine fuming No spot
Pure Bromadiolone	One (Blue)	92	Nil	2(53,39)
Suspected soil	One (Blue)	92	Nil	2(53,39)
Pure soil	No	No	Nil	No

Table.5 hRf value solvent system B extracted in DMF

sample	Observation in Sun light no spot	hRf	UV/ Long and short wavelength	Iodine fuming
Pure Bromadiolone	One(Blue)	90	Nil	2(10,47)
	One(Brown)	78		
Suspected soil	One(Blue)	90	Nil	2(10,47)
	One(Brown)	78		
Pure soil	One(Brown)	75	Nil	2(20,49)

Table.6 hRf value solvent system E extracted in Acetone

sample	Observation in sunlight on spot	hRf	UV/Long and short wavelength	Iodine fuming
Pure Bromadiolone	one(Blue)	96	Nil	1(72)
Suspected soil	One(Blue)	96	Nil	1(72)
Pure soil	No	No	Nil	No

Table.7 hRf value solvent system E extracted in Hexane

sample	Observation in sunlight no spot	hRf	UV/Long and short wavelength	Iodine fuming
Pure Bromadiolone	One(Blue)	90	Nil	3(10,47,52)
Suspected soil	One(Blue)	90	Nil	3(10,47,52)
Pure soil	No	No	Nil	No

Table.8 hRf value solvent system E extracted in DMF

Sample	Observation in sunlight spot	hRf	UV/ Long and Short	Iodine fuming spot
Pure Bromadiolone	One Blue	92	Nil	2(27,42)
Suspected soil	One blue	92	Nil	2(30,40)
Pure soil	No	No	Nil	2(34,41)

Table.9 hRf value solvent system G extracted in Acetone

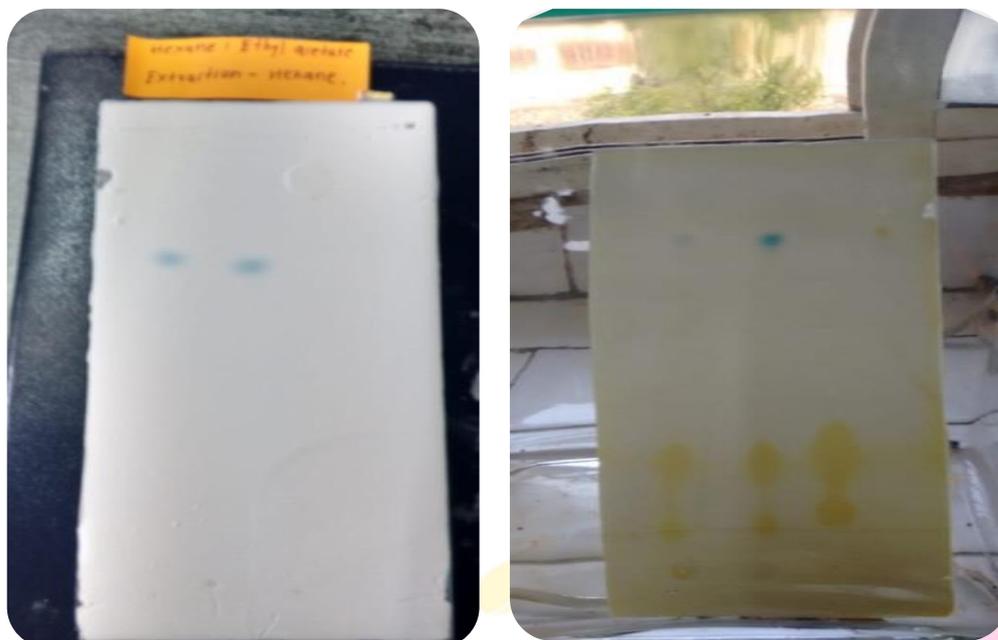
sample	Observation in sunlight no spot	hRf	UV/Long and short wavelength	Iodine fuming no spot
Pure Bromadiolone	One(Blue)	98	Nil	2(47,32)
Suspected soil	One(Blue)	98	Nil	2(47,32)
Pure soil	No	No	Nil	1(42)

Table.10 hRf value solvent system G extracted in Hexane

sample	Observation in sunlight no spot	hRf	UV/Long and short wavelength	Iodine fuming no spot
Pure Bromadiolone	One (Blue)	92	Nil	3(39,32,24)
Suspected soil	One(Blue)	92	Nil	3(39,32,24)
Pure soil	No	No	Nil	1(11)

Table.11 hRf value solvent system G extracted in DMF.

Fig.3. Images of TLC



2.Uv-Visible Spectroscopy

Visible spectra of analyzed sample shows maximum absorption at wavelength.UV-spectrum-bromadiolone wavelength 260-310nm.

Observation: The UV-visible spectra of analyzed sample shows maximum absorption at 215.55nm,220.64nm,323nm,316nm,265.13nm,203nm and in angstrone 1.07A, 1.10A, 1.61A, 1.58A, 1.32A,1.01A respectively.Three different solution were tried for the extraction of this sample **Table 12.**Out of these three solvent acetone was found to be the best solvent for the extraction of these sample.

Table 12 results Uv-Visible Spectroscopy.

Sr no	Extraction	Sample	Lambada max (in nanometer)	Absorbance
1	Hexane	Bromadiolone	215.55	1.07A
2	Hexane	Suspected soil	220.64	1.10A
1	Acetone	Bromadiolone	323	1.61A
2	Acetone	Suspected soil	316	1.58A
1	DMF	Bromadiolone	265.13	1.32A
2	DMF	Suspected soil	203	1.01A

Observation: The UV-visible spectra of analyzed sample shows maximum absorption at 215.55nm,220.64nm,323nm,316nm,265.13nm,203nm and in angstrone 1.07A, 1.10A, 1.61A, 1.58A,

1.32A,1.01A respectively.Three different solution were tried for the extraction of this sample **Table 12**.Out of these three solvent acetone was found to be the best solvent for the extraction of these sample.

1.Pure Bromadiolone extracted in acetone

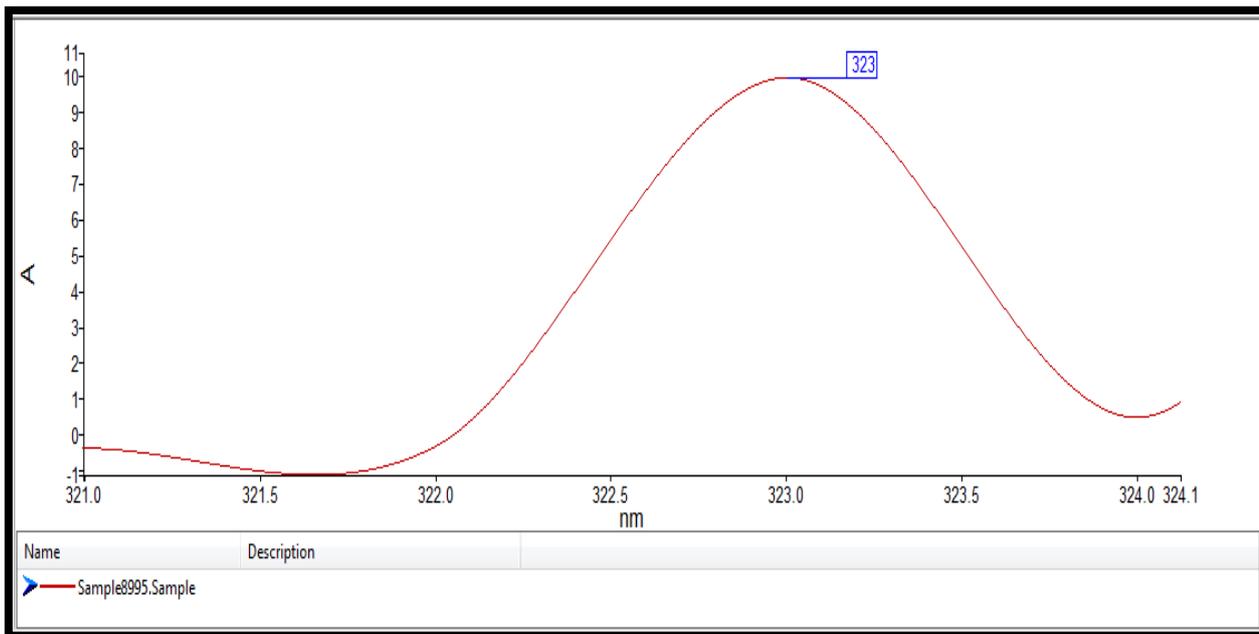


Fig.1.(a) Uv-vis spectrum of pure bromadiolone extracted in acetone.

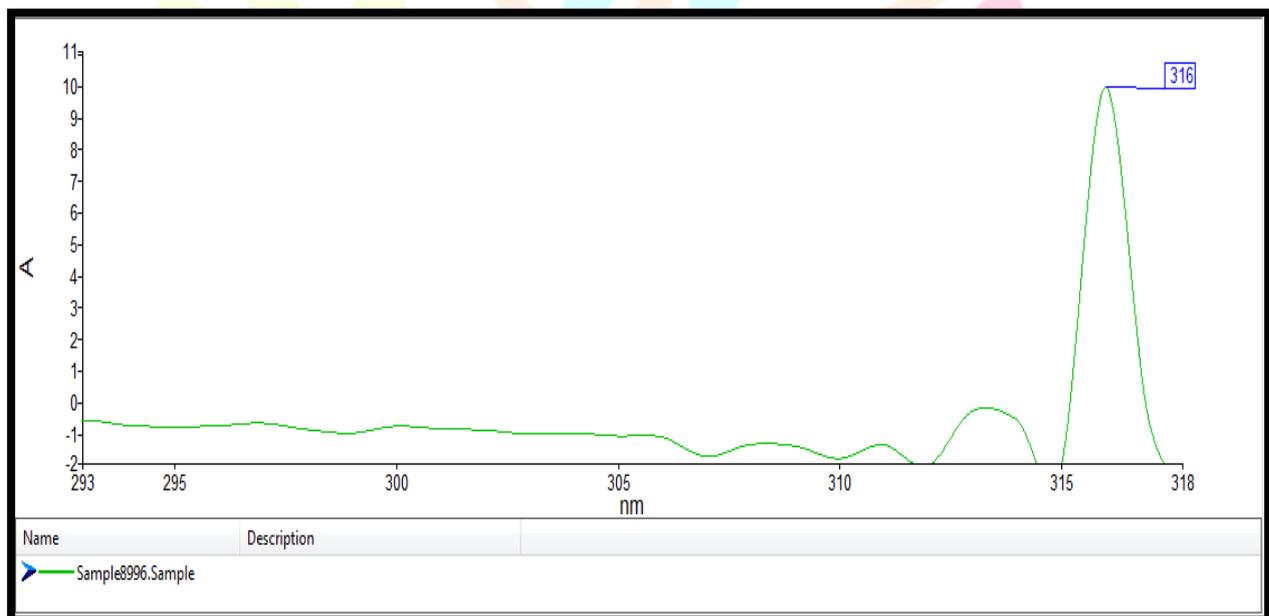


Fig.2 (b) Uv-vis spectrum of Suspected soil extracted in acetone.

3.FT-IR

Hexane Slight change in IR spectra have taken place in the sample under different thermal and there are no changes in sample under different extraction solvent used.We got the values at wave number sample bromadiolone $C_{30}H_{23}BrO_4$.3657 and 3226 cm^{-1} belongs to the -OH(symmetric stretching) and 2834 cm^{-1} belongs to the aldehyde(bond to H atom) and 1017 and 1116 cm^{-1} (-C-O &-C-C-) saturated alkenes.

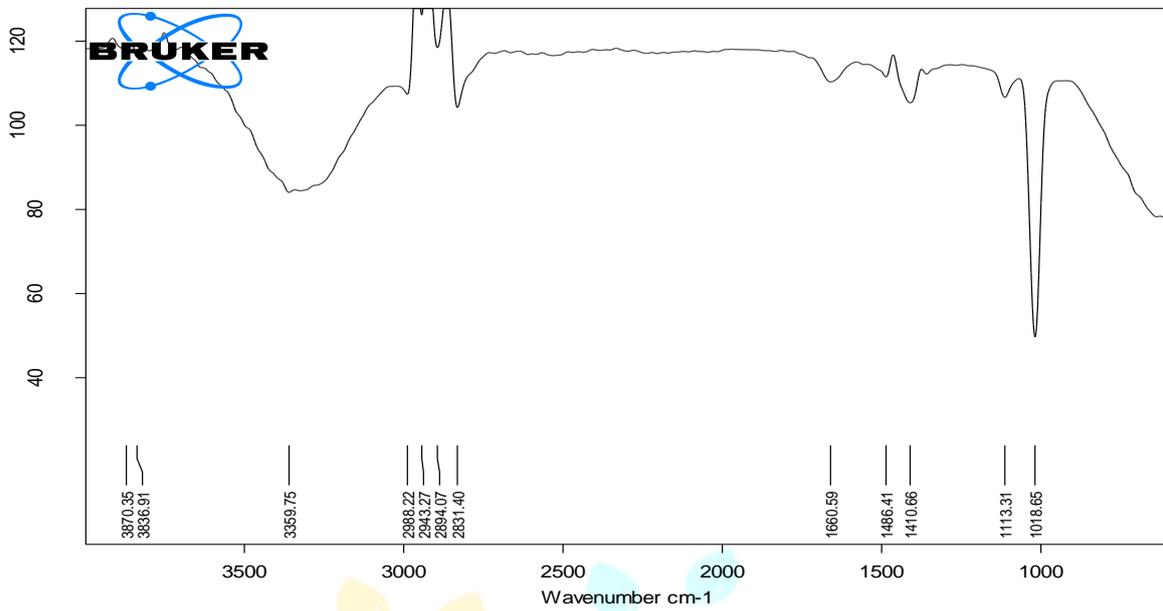


Fig 3. IR spectra of bromadiolone extracted in Hexane

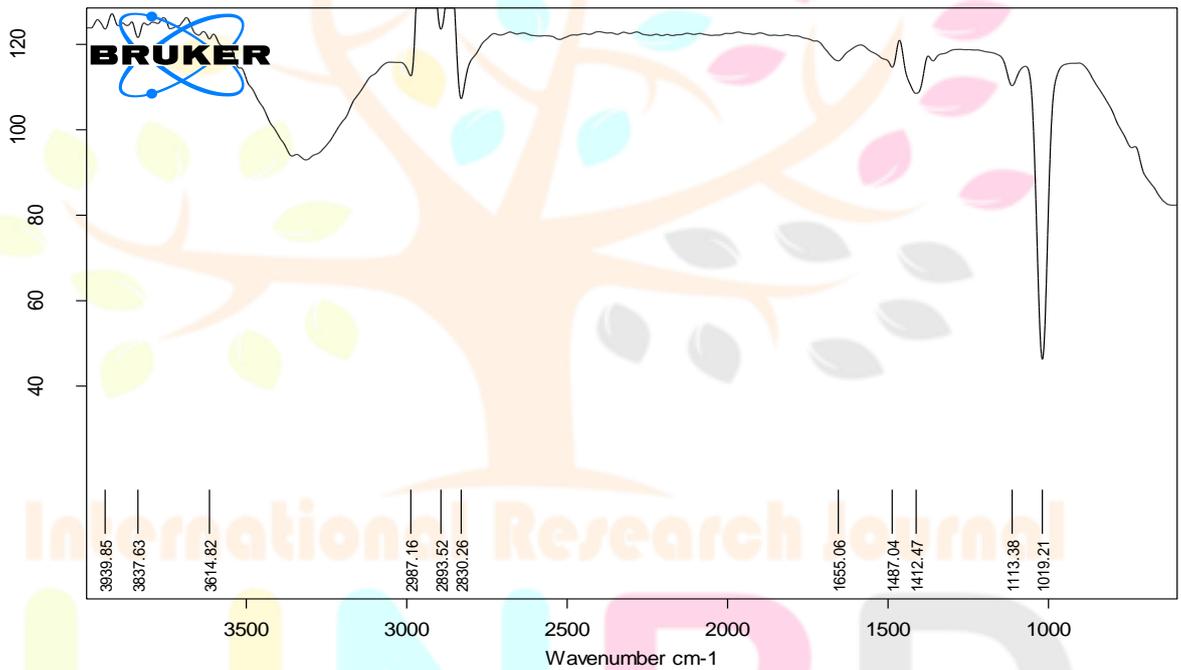


Fig.4 IR spectra of suspected soil extracted in hexane

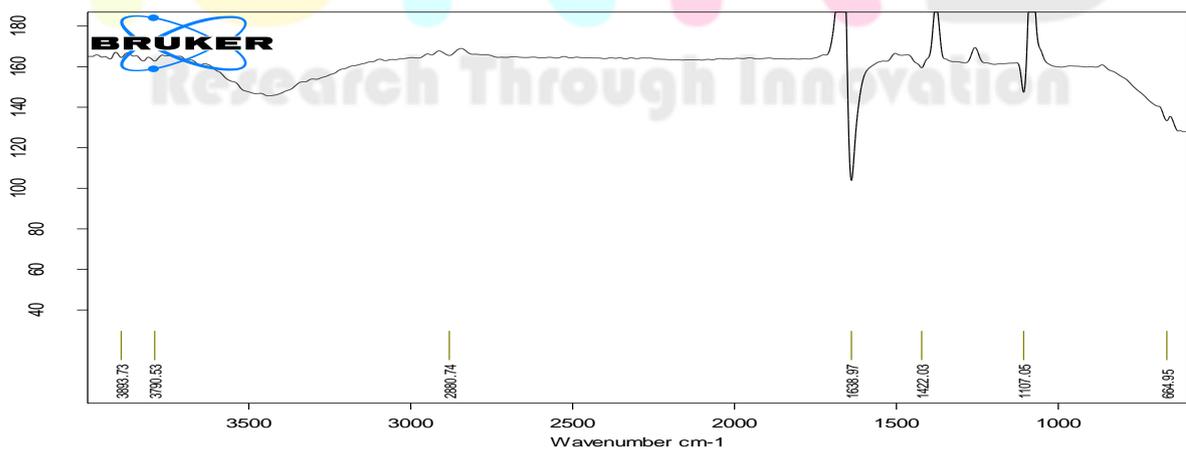


Fig.5 IR spectra of bromadiolone extracted in DMF

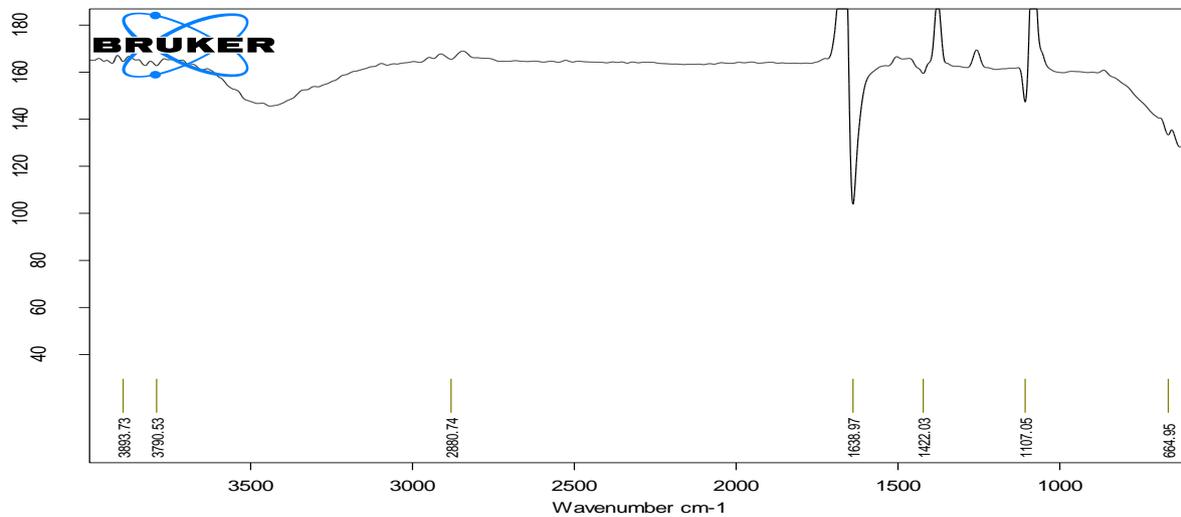


Fig. 6 IR spectra of suspected soil sample extracted in DMF

Hexane and DMF was found to be the best solvent for extraction of these samples (Fig.4 & Fig 6)

Conclusion

Rodenticides are widely available in the market in very cheap price, rodenticides most commonly use in agriculture to control the rodents, it use to kill rats, mice, other rodents and predators is an intense mammalian poison, and it is used in several countries. It is high toxicity to rodents and human, this is single dose anticoagulant rodenticides, this damage to the central nervous system can cause paralysis, convulsions and death. It is increasiment being used by people for committing suicide and even homicide. This study was conducted for the purpose of forensic analysis of traces of bromadiolone poison from soil at site where biological flesh sample buried. Thin layer chromatography (TLC), Uv-Visible spectrophotometer and FT-IR, was employed and results indicate that it could be very helpful as a screening or traces procedure to detect this poison in soil sample and it will be very helpful for forensic investigation process.

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