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Determination of Tropane Alkaloid (Atropine, Anisodamine, Homatropin and Scopolamine) In Seed of Wheat, Corn, Soybean and Linseed

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***Abstract*—The present research work was carried out to determination of tropane alkaloid (atropine, anisodamine, homatropine and scopolamine) in seed of wheat, corn, soybean and linseed. The Tropane alkaloids are toxic for living organisms was subjected to phytochemical and anthelminaic screening. The results are summarized as below. The past study of (Yoshiyuki sawabe et al. 2011) retention time (Rf) of atropine 4.136, regression coefficient (R2) is 0.9987 and limit of detection (LOD) 0.1µg/kg. Also the retention time (Rf) of scopolamine 12.095, regression coefficient (R2) 0.999 and limit of detection (LOD) 0.0210.1µg/kg.**

The limit of detection (LOD) was in the range of 0.02ng/kg of scopolamine in maize, wheat, soybean and linseed. The range of regression coefficient (R2) scopolamine in samples 0.9942, 0.9996, 0.9977 and 0.9988 in soybean, wheat, linseed and maize respectively. The high concentration of scopolamine is 724.082 in wheat, and second number concentration value is 235.632 in soybean. Middle value of scopolamine is 0.183 in linseed and least concentration of scopolamine is 0.146 in maize.

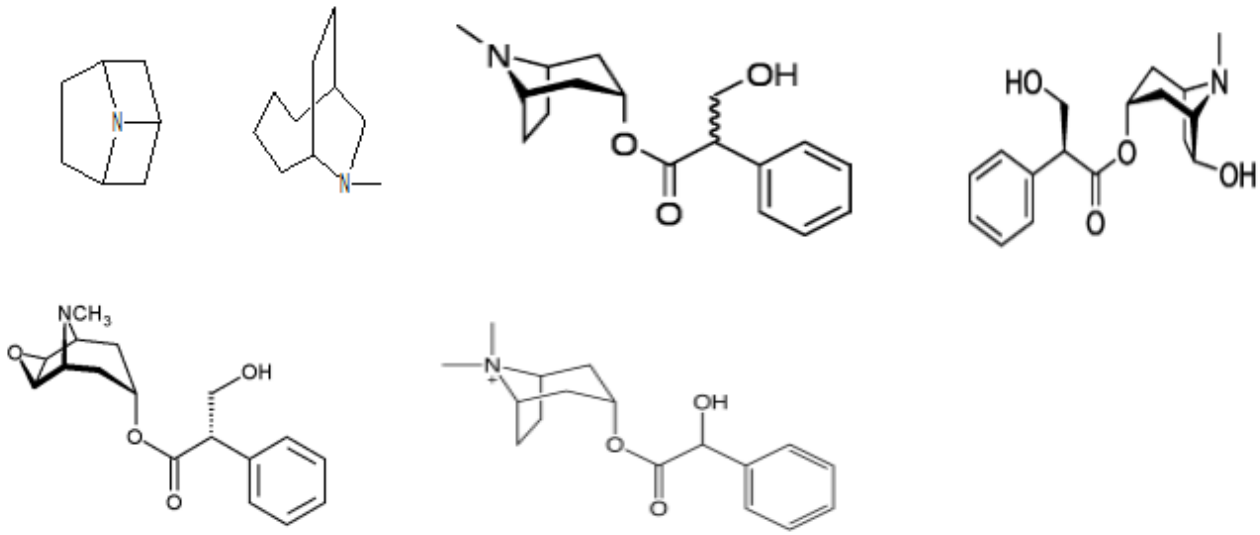
***Keywords*— tropane alkaloids; in seed of wheat, corn, soybean and linseed, HPLC.**

I. INTRODUCTION

Tropane alkaloids are the very important class of alkaloids. These secondary metabolites (Tropane alkaloids) which contain an almost one group of Tropane ring in their chemical structure. Tropane alkaloids are a bicyclic nitrogenous organic compound. That group of alkaloids derived from it known as Tropane alkaloids. In Tropane alkaloids are plant and animal have the one nitrogen containing chemical ring structure. They are very reactive like alkali, and pharmacologic activity. These alkaloids are a symbol of a very large group of medical application compounds that having the well well-known drugs like the (opiates). After the Tropane alkaloids subgroup of the alkaloids is the alkaloid amines. Amine alkaloids are divided into the three major pharmacologic groups [1]. The first groups of alkaloid amine are highly anticholinergic activity of Tropane alkaloids it is also known as the bicyclic (belladonna alkaloids) alkaloids. Second the stimulant alkaloid amines and third are the hallucinogenic alkaloid amines. Tropane alkaloid mainly affect the central nervous system of living organism, as well as spinal cord which control the many direct and indirect body functions also nerve cells of the brain and the behavior of living things. The effect of Tropane alkaloid may also have an effect on the auto mix nervous system. This includes the heartbeat, breathing, circulation and regulation of internal organs. Tropane alkaloids mostly present naturally in many member of the plant family like solanaceae [2, 3]. Tropane alkaloid and Glycoalkaloid are undesirable substance in food. Scopolamine was used from 1940 to 1960 as a pain killer forms others in labor. But these did not stop the pain. But simply removing (eliminated the) memory of pain by attacking the brain functions which is responsible for self-awareness and self-control for few minute of time. These alkaloids were very active ingredient in asthmador. These were counter smoking research marketed in the 1950 and 1960 demanding to combat asthma and bronchitis. The Tropane alkaloid and Glycoalkaloid are toxic naturally produce by the family Solanaceae, Nightshade family including (about 100 genera and 300 species) in world wide. The most significant natural Tropane alkaloids are atropine; homatropine, anisodamine and Scopolamine the concentration of these alkaloids have been found particularly in Poaceae, Fabales, LinaceaeDaturastramonium and Daturaferox (4).

The growing interest in natural toxins produced by fungi and plants in recent years due to its toxicity and its impact on food and feed safety conditions Tropane alkaloids and that Glycoalkaloid (GAs) Back to toxins produced naturally by the Solanaceae family, which includes more than 100 genera and 3,000 species of plants found in all parts of the world (the European Food Safety Authority (EFSA) 2008). Found high concentrations of alkaloids eggplant especially in Datura

stramonium (Jimsonweed), Datura ferox (Long Spined thorn apple), Datura anoxia (Thorn Apple or Moonflower), Mandrake belladonna (deadly nightshade), Haosseamos Niger (black henbane or stinking nightshade), Brugmansia (angel trumpet) and Solanum NIGRUM. These plants are weeds of cultivated fields, wild, courtyards, and other disturbed habitats. They produce varying amounts of Tropane alkaloids, gas and all parts of the plant, especially the seeds, and can be toxic. Grain and seed business in large quantities, such as wheat, barley, soybeans, linseed, corn, crops may be Bazngana contaminated by non-grain impurities that coexist with the crop to be reaped [5,6]. Tropane is a bi-cyclic at least one nitrogenous group containing organic compound. It is mostly identified for a group of alkaloids derived from it known as Tropane alkaloids. Tropane alkaloids arise in plant families Erythroxylaceae (including coca) and Solanaceae (Tomato, Potato, Deadly Nightshade, Datura, Henbane and Mandarke). They have nitrogen bridge in between C-1 and C-5, asymmetric carbon atoms. Mostly Tropane alkaloids are optically inactive due to its symmetry of carbon atom [7].
This can stop the death rattle of dying patients. Atropine is a racemic mixture of l-hyoscyamineand-hyoscyamine with most of its physiological effects due to the presence-hyoscyamine. The atropine is a compound of tropane alkaloid which can extract from deadly nightshade (Atropabelladonna), mandrake (Mandragoraofficinarum), jimsonweed (Datura stramonium) and other plants of the family Solanaceae. It is a competitive antagonist for the muscarinic acetylcholine receptor (8). Scopolamine is also the compound of Tropane alkaloids which is the secondary metabolites. Scopolamine is also called as hyoscine and levo-diboisine is a Tropane alkaloid having special different drug with muscarnic antagonist (rival) effects on health. It is also known as hyoscine and levo-duboisine is a Tropane alkaloid drug having the muscarinic antagonist effects on health. It is amongst the secondary metabolites of plants in family Solanaceae of plants [9]. Anisodamine is a member of tropane alkaloid which is naturally occurring compound. Anisodamine used as an anti-oxidant that might protect against free radical-induced cellular damage. Anisodamine eventually acts by an improvement of blood flow in the micro-circulation. It is also aα1-adrenergic and anticholinergic receptor agonist used in the treatment of acute circulatory system. Scopolamine is naturally occurring tropane alkaloids which are found in large amount of plants in the family Solanaceae [10].



Figer: Structure of Tropane, Atropine, Scopolamine and Anisodamine.

II. MATERIALS AND METHODS

The research work was planned to evaluate the tropane alkaloids from tropane, atropine, scopolamine, anisodamine and homatropine by High Performance Liquid Chromatography (HPLC). The research work has been done on Zea mays (Maize), Triticum durum (Wheat), and Glycine max (Soybean) and Linum usitatissiumun (Linseed) to determine the toxicity. Preparation of extracts was done at chemistry laboratory Department of chemistry, Comsats and the work related to determination of toxicity were done at Toxicity Aflatoxins laboratory, National Institute for Agriculture and Biotechnology, (NIAB) Faisalabad. For the conduction of research following strategies and layout were adopted.

Materials

Chemicals and reagents

Chemicals used in the present research work are given as:
Tri ethyl ammonium buffer, Ammonium hydroxide or Ammonium format, Acetonitrile Ammonium acetate, Distilled water, Chloroform, HPLC-grade Methanol, Formic acid
Magnesium sulphate, Sodium Chloride, Sodium citrate, NaCl and HCl, Atropine standard solution, Scopolamine standard

solution, Homatropine standard solution, Anisodamine standard solution

Instruments Conditions

The HPLC analysis was carried out on a HITACHI Model: UV Detector L-2400 HPLC system (Japan) equipped with a Eurospher C18 column (25 cm × 4 mm i. d., RP) and a UV detector. Elution was monitored at 210 nm. Isocratic elution with a mixture of tri ethyl ammonium phosphate buffer (30 Mm, pH 6.2) and acetonitrile (75:25) at a flow rate of 1.0 ml/min was selected to achieve maximum separation and sensitivity. Hyoscyamine and scopolamine hydro bromide were obtained from Sigma-Aldrich. The injection volume was 50 µL. The elution was achieved using mobile phase composed of 10mM ammonium format in water/acetonitrile (90:10, v/v, solvent A) and methanol/acetonitrile (50:50, v/v, solvent B) mixed in the ratio 20:80 (v/v, A: B). The assay took 16 min and was performed at 45 (±5) °C with a flow rate of 1ml/min. The auto sampler temperature was kept at 40 (±2) °C and the injection volume was 10µl.

Preparation of reagents

All the reagents were prepared accurately and with great care to ensure good quality and enhanced accuracy in the experimental results

Ammonium acetate solution

To prepared the ammonium acetate 0.1mol/L of the standard stock solution. Take the 7.7g of ammonium acetate to dissolve in 1000ml distilled water. Standard solution was diluted 10mM/L, takes the 10 ml of standard stock solution and dissolved in 100mL distilled water.

Preparation of HCl Solution

To prepared 0.1mol/L solution of HCl. Take 3.6g of HCl to dissolve in 1000ml distilled water. Now further dilute 10mM/L, the 10ml of standard stock solution and dissolved in 100mL distilled Water

Reference standard solution

The reference standard were prepared Acetonitrile- 10mM/L Ammonium acetate (8:2 v: v). Take the 80ml of Acetonitrile and 20ml of ammonium acetate dissolved. Filtrate the mobile phase solution with 0.45 brine filter paper and adjusted to pH 5.0, used as the mobile phase. The stability of the baseline and shape of the peaks were poor and retention time of atropine and scopolamine were too short at 20 and 30mM/L. and if the concentration of mobile phase is 5mM/L the retention time is too long.

Standard Solution

The standard solutions (10g/l) of tropane alkaloids (atropine, homatropine, anisodamine and scopolamine), were prepared individually in methanol and kept at 25°C. From these individual stock solutions, an intermediate standard mixture (100000ng/ml) of all four alkaloids was prepared. The curves were plotting the ratio of the signal intensities (peak area) of the analyte and the IS (scopolamine) versus the analyte concentration.

Pre-treatment and storage of samples

The seed samples were washed thoroughly with tap water to remove any wastes and dust particles. The remaining water in the plant material was removed using paper towel by pressing on them gently. The dried the sample in shady place then put the sample in oven at 30-35C for 2hr to remove the moisture form then The seed samples of Zea mays (Maize), Triticum durum (Wheat), Glycine max (soybeans) and Linum usitatissiumun (Linseed) were dried by keeping them in open air under shade for three weeks till constant weight was achieved.

Drying and Grounding of Plant Material

The dried samples were ground to semi-powder form particle size 0.25 using commercial grinder (TSK-949, WestPoint, France) and were stored in air tight polythene bags in refrigerator for further use

Preparation of extracts

The ground samples of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed) were used to prepare extracts. Two different solvent systems were used i.e., HCl and methanol to evaluate the effect of extraction media on the percent yield and the tropane alkaloids capacities of the extracts of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed). Briefly, 1gram of ground material was taken in conical flask followed by the addition of methanol and HCl solution (8:2) (v: v) of each solvent separately

Shaking and centrifuging

The extraction was executed for 10min in an orbital shaker (Gallenkamp, U.K) at ambient temperature. The solution of sample now refrigerator (24ºC) in air tight vials for 5min. The residue from the extracts was separated.

Filtration

This methanol and HCl extraction was filtered with brine filter paper pore size 0.45u. The same procedure was repeated for three times to get maximum amount of extract and all the three extracts were mixed. The sample is ready for HPLC analysis.

III. RESULTS AND DISCUSSIONS

The present research work was carried out to determination tropane alkaloids (atropine, anisodamine, homatropine and scopolamine). All the experimental work was carried out in the research laboratory of Department of Wild Life and Fisheries, GC University, Faisalabad. The extraction of powdered samples was executed by employing two solvent systems i.e. 20% HCl and 80% Methanol by shaking method and the yield of the extracts were calculated. Determine the tropane alkaloids in sample

High Performance Liquid Chromatography (HPLC)

The undertaker study on the determination of atropine, scopolamine, anisodamine and homatropine in Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed) was accomplished using liquid chromatography (LC). High Performance Liquid Chromatography (HPLC) setting were optimized using a syringe load with 10u (company Name), pressure of column is 1.8ml/min and UV detection at 210nm to facilitate experiments for each tropane alkaloid. Direct injection was done. Determination of scopolamine and atropine in food using solid phase extraction to obtain the preparation of a pentaflurophenylpropyl column to improve the peak sharp This column having unique selectivity because of the presence of pentaflurophenylpropyl, its hydrophobicity, and the specific interaction of this compound with aromatic ring and hetero ring compounds. This column has particular advantages because a U-sharp relationship is formed between the concentration of organic solvents and retention time for some compound. A mixture of acetonitrile and ammonium acetate solution was used as the mobile phase of HPLC. The concentration and pH of ammonium acetate solution, and the acetonitrile concentration in the mobile phase, were examined. pH of ammonium acetate solution in the mobile phase is pH 5.0 examined. The stability of the baseline and the shape of the peaks were poor and the retention (RT) time of scopolamine and atropine is too short at 20-50 mmol/L. The retention time (RT) is too long at 5mmol/L. Thus I decided to use 10mmol/L ammonium acetate and adjusted to pH 5.0 [11].

Maintain pH

Adjust the pH of mobile phase ammonium acetate. The resolution of atropine and scopolamine is incomplete at pH 3.0 and 4.0, but at the 5.0 pH give the above then 90% resolution. Therefore I decided to use 10mmol/L ammonium acetate and adjusted to 5.0

Different mobile phases were evaluated for separation of the atropine enantiomers as well as the tropane alkaloids. Efficient resolution of the atropine enantiomers was determined using a mobile phase consisting of methanol, acetic acid and tri ethylamine. Based on the mobile phase used by these groups with different ratios of acetic acid and tri ethylamine in methanol (100%) was evaluated. Elution with 10mmol ammonium format in a water/acetonitrile and methanol/acetonitrile mixture provided better, 13r results, giving good chromatographic resolution of the different analyte and higher sensitivity [12].

This procedure was applied to the analysis of plant material, which necessitated extraction and purification of tropane alkaloids prior to HPLC analysis. Conditions for these preliminary steps were studied and check the extraction under reflux did not modify the chemical structure of alkaloids, controls were performed and internal standard alkaloids added to plant powder sample indicated that these compound were not converted to alkaloids forms, since 92± and 87± of scopolamine and atropine were recovered as original compounds respectively [14]

The chromatograms of the studied commodities are displayed in (Figures 4.1, 4.2, 4.3, and 4.4). it is evident that the resolution of individual compound is very stable with good sensitivity. The limit of detection (LOD) was in the range of 0.1 to 0.02ng/kg of scopolamine and atropine respectively. The Co-extracts did not create any interference and single peaks of the desired compound were eluted with zero tailing. The data in Table (4.1) highlight the retention time with regression coefficient (R2) and linear equation. The range of regression coefficient (R2) atropine and scopolamine in reference standard is 0.9987 and 0.9999 respectively.

Table 4.1 Retention time of Scopolamine and atropine in standard solution

TABLE I

Compound Name	Retention Time (Rf)	Liner Equation	Regression coefficient (R^2)	Limit of detection(LOD)
Atropine	4.135	y = 8180.8x - 133.49	0.9987	0.1µg/kg
Scopolamine	12.095	y = 9715.6x + 248.88	0.9999	0.02 1µg/kg

Table 4.2 Retention time of atropine and scopolamine in samplesolution (Maize, Wheat, Soybean and Linseed)

	Food and feed	Retention Time (Rt)	Liner Equation	Regression coefficient (R^2)	Limit of Detection (LOD)
Compound	**Soybean**	7.359333	y= 171092x - 25736	R2 = 0.9942	**0.02 1ug/kg**
	Wheat	7.403	y = 40582x + 1237.5	R2 = 0.9996	
	Linseed	7.441333	y = 44381x - 3316.5	R2 = 0.9977	
	Maize	7.462333	y = 42763x - 1341.4	R2 = 0.9988	

Table 4.3 Quantitive values of atropine, scopolamine and total alkaloid

Compound Name	Food and feeds	Concentration
Atropine	Maize	
	Wheat	
	Soybean	
	Linseed	
Scopolamine	Maize	0.14644 µg/kg
	Wheat	724.08288 µg/kg
	Soybean	235.62 µg/kg
	Linseed	0.18380 µg/kg

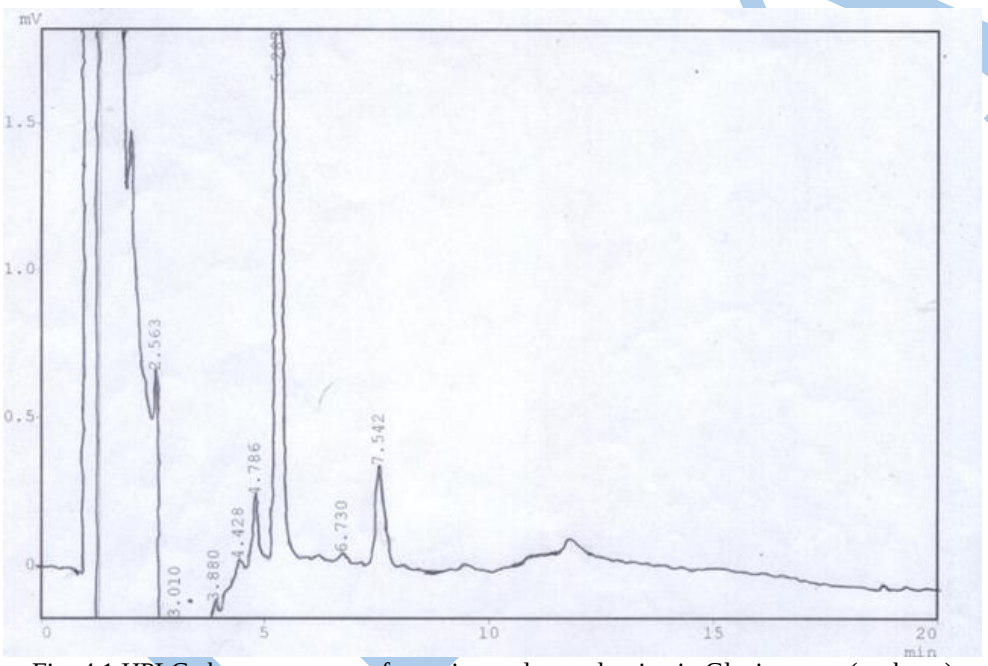


Fig. 4.1 HPLC chromatograms of atropine and scopolamine in Glycine max (soybean)

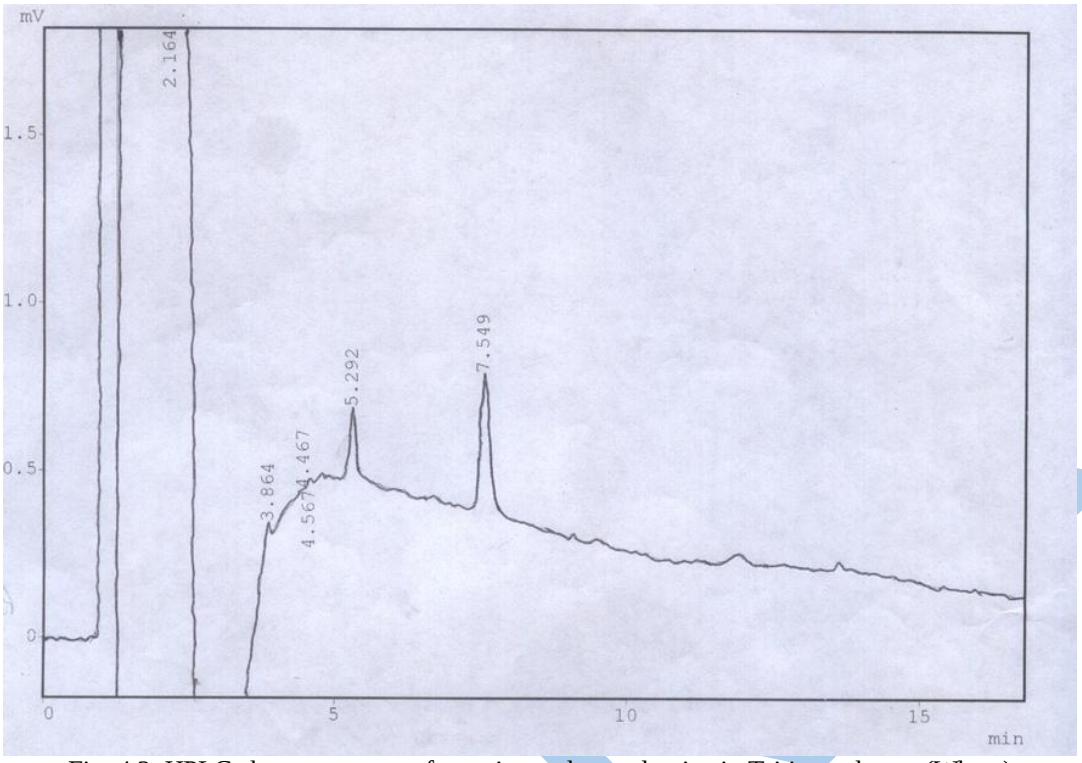


Fig. 4.2: HPLC chromatograms of atropine and scopolamine in Triticum durum (Wheat)

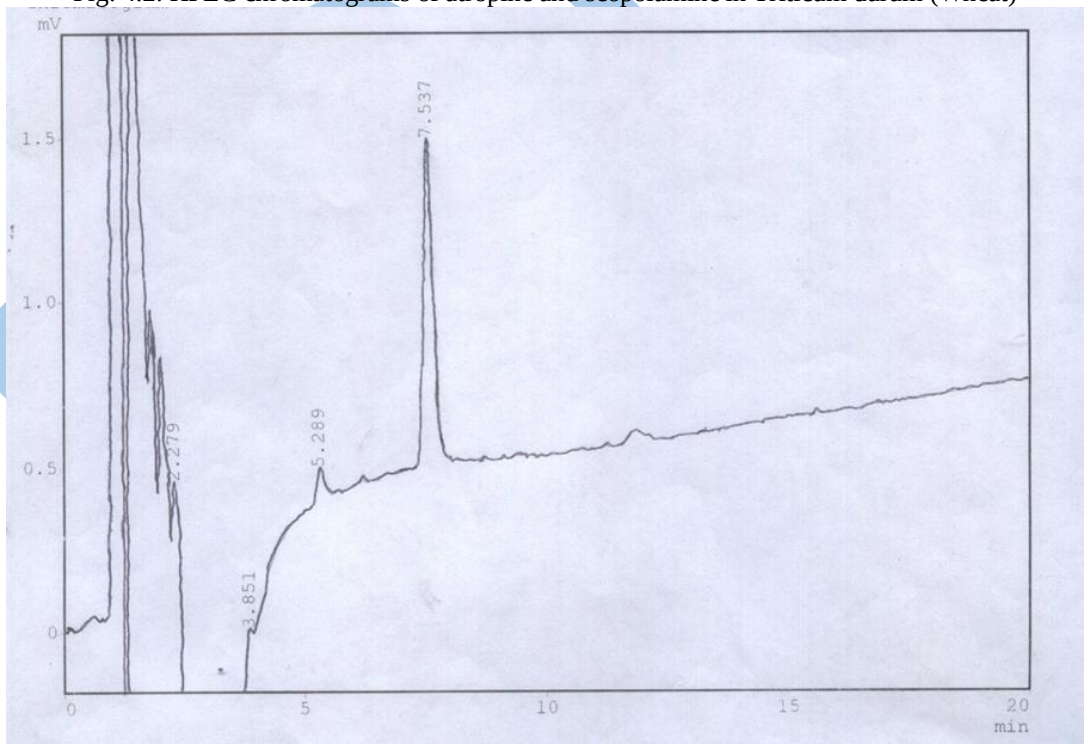


Fig. 4.3: HPLC chromatograms of atropine and scopolamine in Linum usitatissiumun (Linseed)

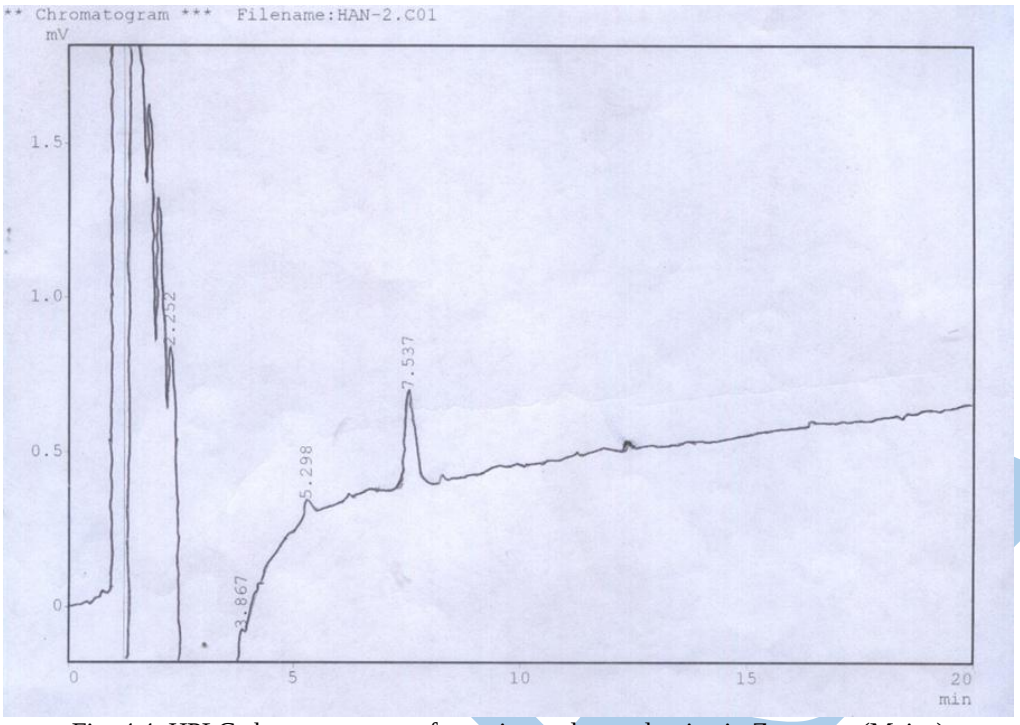


Fig. 4.4: HPLC chromatograms of atropine and scopolamine in Zea mays (Maize)
Figer 4.1-4.4. HPLC chromatograms of atropine and scopolamine in soybean, Wheat, Linseed, and Maize.

Discussion

After getting information of standards the unknown samples of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed), where analyzed by RP-HPLC in isocratic mode. The chromatograms of samples are displayed in (Figures 4.1, 4.2, 4.3, and 4.4). The limit of detection (LOD) was in the range of 0.02ng/kg of scopolamine in maize, wheat, soybean and linseed. The Co-extracts did not create any interference and single peaks of the desired compound were eluted with zero tailing. The data in Table (4.2) highlight the retention time with regression coefficient (R2) and linear equation. The range of regression coefficient (R2) scopolamine in samples 0.9942, 0.9996, 0.9977 and 0.9988 in soybean, wheat, linseed and maize respectively is show in Table (4.2). The base-line and resolution of extracts were excellent and comparable with working solutions of reference standards. The concentrations of scopolamine were calculated using linear equation (y=ax-b). The concentration of extracted sample of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed) is given in Table 4.3.

The value of Tropane alkaloid shows in Table (4.3) that the high concentration of scopolamine is 724.082 in wheat, and second number concentration value is 235.632 in soybean. Middle value of scopolamine is 0.183 in linseed and least concentration of scopolamine is 0.146 in maize.

Table 4.2 Retention time of atropine and scopolamine in sample solution (Maize, Wheat, Soybean and Linseed)

Table 4.3 Quantitive values of atropine, scopolamine and total alkaloid

Total Concentration of Tropane alkaloids

Usually the alkaloids compounds of the plants were responsible for their toxicity capacities. Total alkaloid contents were influenced by different solvents used. Total alkaloids contents of the seed extracts of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed) were determined by using JP 15 method. The HCl/methanol was very sensitive for the alkaloids compounds present in plant extracts. Usually it did not interact with the other.

The scopolamine and atropine analyses conducted during research work showed good precision and accuracies repeatable. The results presented in the Table 4.3 show the total alkaloids contents of the seed extracts of Zea mays (Maize), Triticum durum (Wheat), Glycine max (Soybean) and Linum usitatissiumun (Linseed). The concentration range Tropane alkaloids from 0.14644-724.08288 µg/kg of the dry plant material. The maximum concentration (724.0828 µg/ kg) was obtained from the wheat seed extract, while the minimum Tropane alkaloids concentration (0.146444 µg/kg of dry weight. was obtained from Zea mays (Maize), seed extract. The statistical analysis showed the significant differences ($p < 0.05$) in the different extracts.

It was observed that data presented correlate with the work of [11]. According to their study, the Tropane alkaloid concentrations were in the range of 84.5-1033 µg/kg of dry matter. The results of our study suggested that the Tropane

alkaloid were very active regarding toxic capacities and could be exploited for use in food and pharmaceutical.

Regression coefficient (R2)

The regression coefficient of each sample has little difference. In soybean having regression coefficient is 0.9792. Wheat sample having accurate regression coefficient that is 0.9996 Sample of linseed have low regression coefficient as compared to other sample 0.9977. Last sample of maize regression coefficient is 0.9988 values is show in Table 4.2.

Conclusion

Alkaloids in food are recognized as poisoning risks to man and animal's and the current interest of the scientific community in the substances in justified from scientific, food safety and regulatory points of view. The HPLC method for the rapid determination of tropane alkaloids at low concentration levels enables fast, reliable selective and sensitive quantification of the major alkaloids, with good accuracy, precision and linearity (R2=0.9942-0.9996). A simple extraction and clean up procedure, short analytical run time and (LOD) together make method suitable for routine application in various type of seed. The first reported on the simultaneous analysis of tropane alkaloids in food. Four of the target toxins were detected in food sample. It were reported that extraction yield depends upon the nature of plant material to be extracted, solvent systems. Therefore, it is imperative to develop an appropriate extraction method to extract maximum quantity of Tropane alkaloids before its exploitation for possible applications in food conditions [11].

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