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Determination of Imazamox and Imazethapyr Herbicide Residues in Soybean oil

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ABSTRACT

A easy, sensitive and inexpensive method was evolved the usage of solid-phase extraction, together with high performance liquid chromatographic method with UV detection for determination of imazamox and imazethapyr residues in soybean oil samples. The evaluated parameters consist of the extracts via C_{18} cartridge, using hydrochloric acid, hexane, methanol, methylene chloride and acetonitrile solvents. The method becomes established the use of soybean oil samples spiked with imazamox and imazethapyr at different fortification levels (0.01 and 0.1 µg/mL). Average recoveries (using each concentration six replicates) ranged 87-94%, with relative standard deviations less than 3%, calibration solutions concentration in the range 0.01-5.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.003µg/mL and 0.01µg/mL respectively.

Key words: HPLC-UV, Imazamox, Imazethapyr, solid-phase extraction and soybean oil.

INTRODUCTION

Imazamox is the common call for(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-three-pyridinecarboxylic acid [1]. Imazamox is a member of theimidazolinone elegance of herbicides that also consists of imazapic, imazapyr, imazethapyr, imazame thabenz, and imazaquin. it's miles used for control of maximum annual and perennial broadleaf weeds and grasses, woody species, and riparian and emergent aquatic weed species [2].

Imazamox is formulated each as an acid and as an isopropylamine salt. Uptake of imidazolinone herbicides is in general through the foliage and roots. The herbicide is then translocated to meristematic tissue (buds or areas of boom) by way of the xylem and phloem where it inhibits acetohydroxy acid synthase [AHAS; also known as aceto lactate synthase (ALS)], an enzyme worried inside the synthesis of three crucial amino acids (valine, leucine, and isoleucine). Those amino acids are required for protein synthesis and cell increase. Imazamox as a result disrupts protein synthesis and interferes with cellular boom and DNA synthesis, inflicting the plant to slowly die. AHAS isn't present in mammals, birds, fish, or invertebrates making it specially toxic to flora [3,4].

Imazethapyr is an imidazole compound used as a selective herbicide. it's miles carried out preplant integrated, preemergence, at cracking, and postemergence [5]. The compound controls weeds through decreasing the tiers of 3 branched-chain aliphatic amino acids, isoleucine, leucine and valine, thru the inhibition of aceto-hydroxy acid synthase, an enzyme commonplace to the biosynthetic pathway for these amino acids. This inhibition reasons a disruption in protein synthesis which, in turn, results in interference in DNA synthesis and cellular boom [6,7]. The compound is used to manipulate grasses and broadleaved weeds such as barnyard grass, crabgrass, cocklebur, panicums, pigweeds, nightshade, mustard, smartweed, velvetleaf, jimsonweed, foxtails, seedling Johnson grass, lamb squarters, morning glory and others. Tolerant vegetation consist of soybeans, peanuts, dry and fit to be eaten

beans, peas, alfalfa and imidazolinone resistant/tolerant corn [8,9]. Additional research is being performed on other leguminous vegetation. Imazethapyr comes in aqueous pay attention formulations. it is also formulated and can be blended with different herbicides [10].

Diverse methods had been defined for the determination of those residues, the usage of stable-phase micro extraction (SPME) Supercritical fluid extraction (SFE) and liquid – liquid extraction. However, not one of the posted researches so far has reported the simultaneous analysis of Imazamox and Imazethapyr in soybean oil.

MATERIALS AND METHODS

Standards, Reagents and samples

The analytical standards of Imazamox (99.5%) and Imazethapyr (98.0%) was obtained from Sigma Aldrich. HPLC grade acetonitrile, orthophosphoric acid and water became bought from Rankem, Analytical grade solvents i.e., hydrochloric acid, hexane, methanol and methylene chloride were brought from Merck Limited and soybean oil become bought from local market.

Standard stock solutions

Accurately weighed 9.88 mg of reference analytical standard of Imazamox in 10ml volumetric flask and the volume was brought up to the mark using acetonitrile. A 10.01 mg of reference analytical standard of Imazethapyr was weighed in a different 10ml volumetric flask and the volume was brought upto the mark using acetonitrile and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample preparation

Representative 25 gram portions of soybean oil fortified with 0.1 mL of working standard solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction procedure

Imazamox

25g of Soyabean oil sample was weighed into a 1-quart Mason jar. 300 mL of extraction solvent **(**40 ml of 1N hydrochloric acid mixed with 1560 mL milliQ water and 2400 mL methanol (40: 60) was added to the sample and blended at medium speed for 5 minutes using a mixer. After blending, approximately 5 g of Celite 545 AW was added to the extract in the jar and swirled to mix. A Celite pad on a 9-cm glass fiber filter paper positioned on a 9-cm Buchner funnel was made by Using vacuum and a 500-mL filtration flask. The mixture was passed through, forming the pad, and collected the extraction solution in the flask. A 50% of the extract was partitioned for the quantification of residues of Imazethapyr. The remaining 50% extract was transferred into a 500-mL round bottom flask and evaporated the extract to approximately 100 mL by Using a rotary evaporator. A 250 mL of acetone was added to the extract in the 500-mL round bottom flask then added approximately 5 g of Celite 545 AW to the extract and swirled to mix. A Celite pad on a 9-cm glass fiber filter paper positioned on a 9-cm Buchner funnel was made by Using vacuum and a 500-mL filtration flask. The mixture was passed through, forming the pad, and collected the acetone in the flask. The filtrate was transferred into a 1000-mL round-bottom flask and evaporated the acetone on a rotary evaporator to a near dryness.

Partition

The residue was dissolved in 1 mL methanol, swirled and sonicated. 4.0 mL of 0.05N hydrochloric acid was added and transferred to the 500-mL separating flask. The round bottom flask was rinsed with 2 x 50 mL of methylene chloride, and transferred each rinse to the same separatory funnel. Partitioned, by shaking vigorously for approximately 30 seconds. Drawn off the lower (methylene chloride) layer into a 1000-mL round-bottom flask. Partitioned the upper (aqueous) layer with 3 x 100 mL methylene chloride, combining the lower (methylene chloride) fractions into the 1000-mL round bottom flask. Evaporate the combined methylene chloride extracts to dryness using a rotary evaporator. Redissolved the residues in 2 mL of methanol followed by 50 mL of acetonitrile, sonicated for approximately 30 seconds, and poured the mixture into a 250-mL separatory funnel. Rinse the 1000-mL round-bottom flask with 50 mL of hexane and combine with the methanol-acetonitrile mixture in the 250-mL separatory funnel.

Partitioned by shaking vigorously for approximately 30 seconds. Allowed the layers to separate, then drawn off the lower (acetonitrile) layer into a 250-mL round-bottom flask. Discarded the upper (hexane) layer. Evaporated the acetonitrile to dryness using a rotary evaporator. Redissolved the residue in 15 mL of 0.05 N hydrochloric acid and sonicated for approximately 30 seconds for solid phase extraction cleanup.

Solid Phase Extraction Clean-up

A C-18 cartridge (500 mg/3mL tube) was prepared by using a Vac-Elut Processing Station and minimal vacuum by washing the cartridge with 3 mL of methanol followed by full-column volumes (approx. 3 mL each) of 0.05 N hydrochloric acid. A 75-mL disposable, fritted reservoir onto the top of the conditioned C-18 cartridge was assembled by using an adapter. Passed the extract from through the C-18 cartridge at the rate of approximately 2-3 drops per second. Discarded the eluate. Rinsed the 250-mL round-bottom flask, reservoir, and C-18 cartridge with a single 5-mL portion of 0.05 N hydrochloric acid, passing the rinse through the cartridge at the rate of approximately 1 drop per second. Discarded the eluate. Removed the reservoir and adapter and washed the C-18 cartridge with 4 full column volumes of 0.05 N hydrochloric acid at the rate of approximately 1 drop per second. Discarded the eulate. Removed the C-18 cartridge. Prepared two SCX cartridges (1000 mg/6 mL tube) using a vacuum by washing each with 5 mL of hexane, 5 mL of methanol and 2 x 5 mL of 1 N hydrochloric acid. Connected the C-18 cartridge onto the top of the two (in tandem) SCX cartridges. Detached and discarded the C-18 cartridge, and connected a 30-mL disposable syringe (plunger removed) onto the top of the tandem SCX cartridges. Washed the tandem cartridges with 5 mL of methanol at a rate of approximately 1 drop per second. Discarded the eluate. Removed the SCX cartridges. Added 30 mL of saturated potassium chloride-methanol to the syringe. Using the syringe plunger, eluted the SCX tandem cartridge system directly into a 100-mL round-bottom flask. Evaporated the saturated potassium chloride-methanol eluate to dryness using a rotary evaporator. Dissolved the residue in 1 mL of methanol, sonicated for approximately 30 seconds, then added 4 mL of 0.05 N hydrochloric acid. Swirled and transferred the solution into a 250-mL separatory funnel.

Added an additional 3 mL of 0.05N hydrochloric acid to the 100-mL round-bottom flask, swirled and transferred to the separatory funnel. Rinsed the round-bottom flask with 2 x 50 mL of methylene chloride, swirled and transferred into the separatory funnel. Partitioned, shaking vigorously for approximately 30 seconds. Drawn off the lower (methylene chloride) layer into a 1000-mL round-bottom flask. Partitioned the upper (aqueous) layer with 3 x 100 mL of methylene chloride, combining the lower (methylene chloride) layers in the 1000-mL round bottom flask. Evaporated the combined methylene chloride fractions to dryness using a rotary evaporator. Dissolved the residue in 1 mL of methanol, sonicated for approximately 30 seconds, then added 4 mL of methylene chloride, swirled and transferred into the separatory funnel. Added 100 mL of methylation reagent to the solution in the 100-mL round-bottom flask, and evaporate to dryness. Add another 20 mL of methanol to the 100-mL round-bottom flask, and re-evaporate to dryness and dissolved the residue in suitable volume of methanol.

Imazethapyr

Partition

The extract was adjusted to pH 2 by addition of 1 M HCl and it was transferred to 500 ml separating funnel, partitioned with 100 ml of methylene chloride thrice. The methylene chloride layer was collected and it was partitioned with 200 ml of pH9 buffer. The aqueous phase was collected and adjusted to pH 2 by using 1 M HCl. Again pH 2 solution was partitioned with 100 ml of methylene chloride thrice. The methylene chloride layer was collected and evaporated to dryness in a rotary vacuum evaporator. The residue was dissolved in suitable volume of methanol. The methanol solution was made up with distilled water in a 25 ml volumetric flask.

Column clean up

The quaternary ammonium amine ion exchange solid phase extraction column was eluted with 10 ml of methanol and 10 ml of distilled water. The aqueous methanol sample solution was passed through the column and the eluant was collected in a 50 ml beaker. The solution pH was adjusted to 2 with 1 M HCl. The pH solution partitioned with 100 ml of methylene chloride thrice in a separating funnel. The methylene chloride layer was collected and evaporated to dryness in a rotary evaporator. The residue was taken in a suitable volume of acetonitrile.

Residues of imazamox in methanol and imazethapyr in acetonitrile were combined together in a round bottomed flask, evaporated to near dryness in a rotary evaporator, the residue reconstituted with suitable volume of acetonitrile for HPLC analysis.

Instrumentation

HPLC-PDA separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (Phenomenex Luna-C18) Column temperature was maintained at 30°C. The injected sample volume was 10µL. Mobile Phases A and B was Acetonitrile and 0.1% ortho phosphoric acid (30:70 (v/v)). The flow- rate used was kept at 1.2 mL/min. A detector wavelength was 254 nm.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.01 and 0.1 µg/mL. Linearity was determined by different known concentrations (0.01, 0.05 0.1, 0.5, 1.0 and 5.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Aliquots of imazamox and imazethapyr samples, control sample solution, extracted solvents and mobile phase solvents were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (**Figure 1 and 2).** Furthermore, the retention time of imazamox and imazethapyr were 5.3 min and 8.9 min (Approximately).

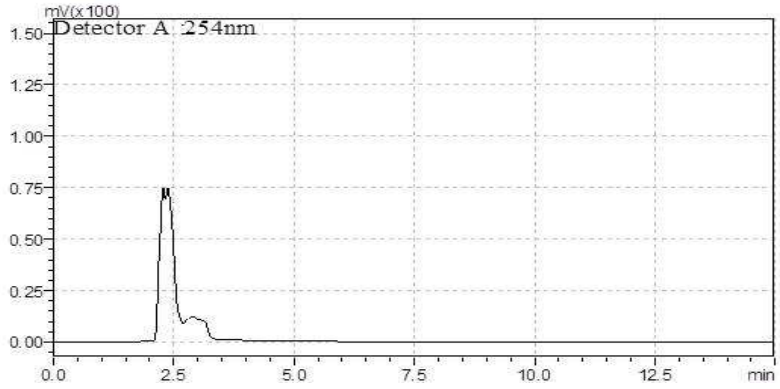

Figure.1. Representative Chromatogram at soybean oil control

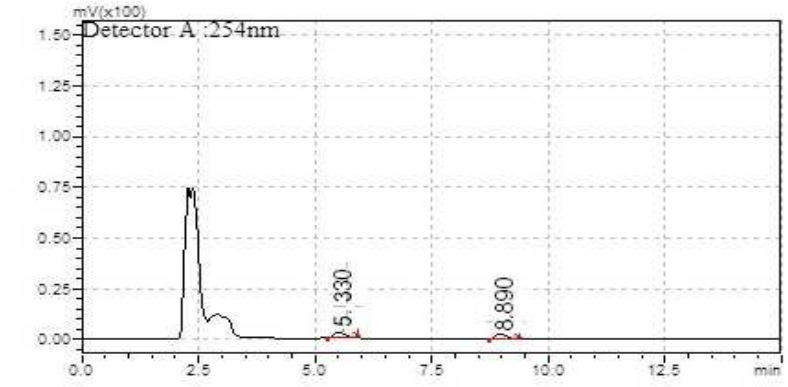

Figure.2. Representative Chromatogram at fortification level of 0.01 µg/mL

Calibration Details

Preparation of Stock solution of reference analytical standard

Accurately weighed 9.88 mg of reference analytical standard of Imazamox in 10ml volumetric flask and the volume was brought upto the mark using acetonitrile. A 10.01 mg of reference analytical standard of Imazethapyr was weighed in a different 10ml volumetric flask and the volume was brought upto the mark using acetonitrile.

Preparation of Calibration solutions

Mixture of different known concentrations of Imazamox and Imazethapyr (5 - 0.01 µg/mL) were prepared in 10ml acetonitrile by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient [11]. Results are presented in **Table 1**. Calibration curve was presented in (**Figure 3**).

Table 1. Serial dilutions of linearity standard solutions

Concentration (µg/mL)	Peak area (µV-sec)	
	Imazamox	Imazethapyr
0.01	121	152
0.05	585	663
0.1	1085	1253
0.5	5443	6224
1	10632	11446
5	50668	61628

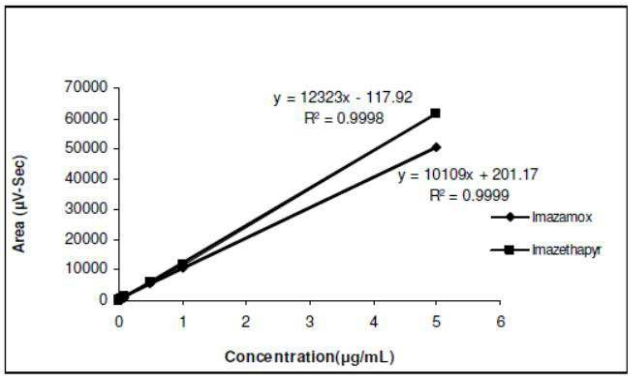

Figure.3. Representative Calibration Curve of Imazamox and Imazethapyr

Recovery-Limit of Determination (LOQ)

Recovery studies in Soyabean oil was conducted by fortifying different concentrations of Imazamox and Imazethapyr standards in the range (0.01 - 0.1 µg/mL). The samples were homogenized, extracted and analysed for residue content, as described in the method of analysis.

The average percent recovery for Imazamox in Soyabean oil was 87±2.05 at 0.01 µg/mL fortification level and 93±1.63 at 0.1 µg/mL fortification levels, respectively.

The Imazethapyr has the recovery percentage 89±1.73 at 0.01 µg/mL fortification level and 94±1.60at 0.1 µg/mL fortification levels, respectively in Soyabean oil. The method has a limit of determination 0.01 µg/mL (LOQ) [12].

Storage Stability

A storage stability study was conducted at -20°C with Soyabean samples spiked with 0.1 ppm of Imazamox and Imazethapyr Samples were stored for a period of 30 days at this temperature. Analysed for the content of Imazamox and Imazethapyr before storing and at the end of storage period. The percentage of dissipation observed during above storage period was only 4% showing for both Imazamox and Imazethapyr no significant loss of residues on storage. Results are presented in **Table 2**.

Table 2. Storage stability Details

Fortified concentration (ppm)	Storage Period (days)	Replication	Recovery (%)	
			Imazamox	Imazethapyr
0.1	0	**R1**	93	92
		R2	94	93
		R3	92	95
		Mean ± S.D	**93±1.17**	**94±1.50**
	30	**R1**	88	91
		R2	89	89
		R3	90	88
		Mean ± S.D	**89±1.00**	**90±1.22**

Calculations

The concentration of acetaminophen in the samples analyzed by HPLC was determined directly from the standard curve.

$Y = mx + c$

Where,

Y = peak area of standard (µV*sec)
m = the slope of the line from the calibration curve
x = concentration of injected sample (mg/L)
c = 'y' intercept of the calibration curve

The recovered concentration or Dose concentration was calculated by using the formula:

$$\text{Recovered concentration or Dose concentration} = \frac{(x-c) \text{ X } D \text{ X } 100}{m \text{ X } P}$$

Where,
m = the slope of the line from the calibration curve
x = sample area of injected sample (µV*sec)
c = 'y' intercept of the calibration curve
D = Dilution Factor
P = Purity of Test item

$$\% \text{ Recovery} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

CONCLUSION

This paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the Imazamox and Imazethapyr residues in soybean oil. The SPE extraction procedure is very simple and inexpensive method for determination Imazamox and Imazethapyr residues in soybean oil. The mobile phase Acetonitrile and 0.1% ortho phosphoric acid showed good separation and resolution and the analysis time required for the chromatographic determination of the sugarcane juice is very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO) guidelines [10]. Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the Imazamox and Imazethapyr residues in different commodities (juice, seed, oil, fruit, and water and soil samples.

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