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ORIGINAL ARTICLE

Quantitative and Qualitative Profiling and Extraction of Acephate Pesticide Residues in Soil and Water using SOLLE and LLE Coupled with Multidimensional Chromatographic Techniques and UV-Visible Spectroscopy

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Abstract:

Acephate is an organophosphate insecticide that has been used to control a wide range of pests, including insects, mites and nematodes. It is commonly used in agricultural settings, as well as for residential and commercial pest control. In water, acephate can undergo degradation through hydrolysis, particularly under alkaline conditions. The half-life of acephate in water can range from a few days to several weeks, depending on factors such as pH and temperature. After degradation, the primary breakdown product is methamidophos which can also exhibit toxicity to aquatic organisms. In soil, acephate can undergo various processes, including degradation, adsorption, and leaching. The persistence of acephate in soil depends on factors such as soil type, organic matter content, pH and microbial activity. In general, acephate has a moderate to high potential for adsorption to soil particles, which can reduce its mobility and availability for degradation. The half-life of acephate in soil can range from a few weeks to several months. A rapid and highly sensitive UV-visible Spectrophotometer were used for the qualitative analysis of Acephate in soil and water. A solution of 500 ppm acephate powder was spiked in soil and water. The analyte was extracted using Sugaring/Salting Out Liquid Liquid Extraction (SOLLE) and Liquid Liquid Extraction (LLE). The solvent used for SOLLE method was Acetonitrile, Hexane and Acetone for LLE. The extract were analysed by Thin Layer Chromatography, Gas Chromatography. Thin Layer Chromatography was performed to find the best solvent system for Acephate. Gas Chromatography and UV-Visible spectrophotometer were used for quantification.

Keywords: Acephate; Thin layer chromatography; Gas chromatography; UV-visible spectrophotometer.

Introduction:

Pesticides are substances that are used to control pests, including weeds.[1] Pesticide includes herbicides, insecticide, nematicide, rodenticide, mollucicide, piscicide, avicide, insect repellent, fungicide, disinfectant and sanitizer.[2] Most of the pesticides are generally intended to use for plant protection against weeds, fungi or insects.[3] It improves the crop livestock yields and quality by controlling pest and plant disease vectors.[4] It can be categorized by target organism like insecticides, herbicides, rodenticides etc., by their chemical structures like organo-phosphate, organochloro and carbamtes. Organochloro pesticide disturbs the Na/K balance of the nerve fiber and allows the nerve to transfer continuously. They are very persistent and stay in the environment for a longer period. Due to the potential bioaccumulation, organochloro is replaced by carbamates and organophosphates.[5] Both inhibit the activity of acetylcho-linesterase, causing weakness of the muscles and paralysis.

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1.1 Organophosphates: Organophosphate insecticide was introduced be Chevron Chemicals in 1973.[6] It is an insecticide used primarily for control of aphids, including leaf miners, caterpillar, sawflies and thrips, on food crops, vegetables and horticulture.[7] They are derived from phosphoric acid which is toxic to vertebrates and also to other animals.[8] They are popularly used more often than other pesticides due to their lack of persistence in the environment since they are chemically unstable in nature and their effectiveness.[9] They are cheaper and easily available in developing countries like India, Nepal and Bangladesh. Poisoning rates in the suicide attempters who attend hospital varies from around 40% to over 80% in many Indian studies and OP compounds available as pesticides are amongst the most common poisons used.[10] Out of the total deaths from self-harm, in the regions of developing countries of Asia, 60% are due to pesticide poisoning and out of the total of 60%, almost 70% accounts to organophosphate.[11]

1.2 Acephate : Acephate (See Figure.1) (O,S-Dimethyl acetylphosphoramidothioate) is an organophosphate insecticide of moderate persistence of about 3-6 days in soil, plants and insects.[12] Although in some soils the half-life may be increased to more than 13–60 days due to variation of properties (physical, chemical and biological) of soils.[13] Acidic nature of soil is responsible for long life span of acephate in soils. After decomposition, acephate converts into metha-midophos (O,S-

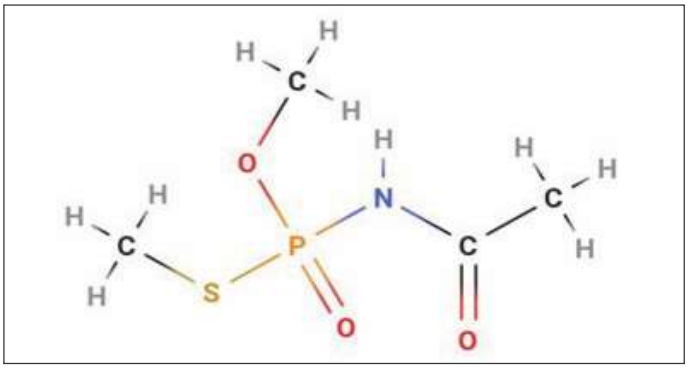

Figure 1. Structure of acephate.

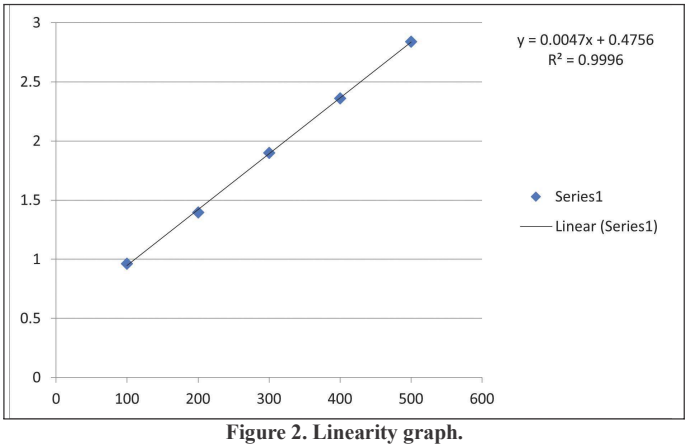

Figure 2. Linearity graph.

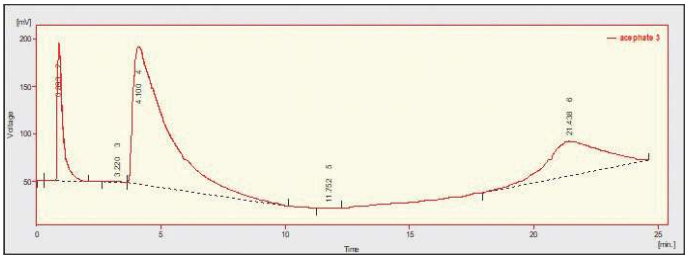

Figure 3. Standard acephate.

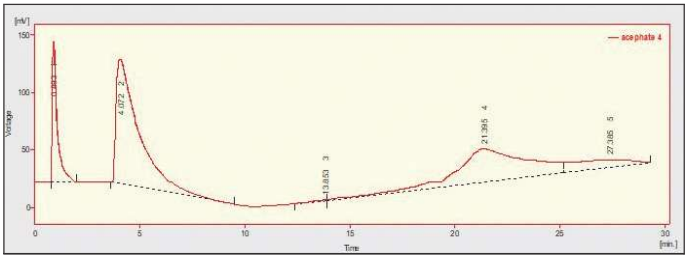

Figure 4. Extracted sample from SOLLE.

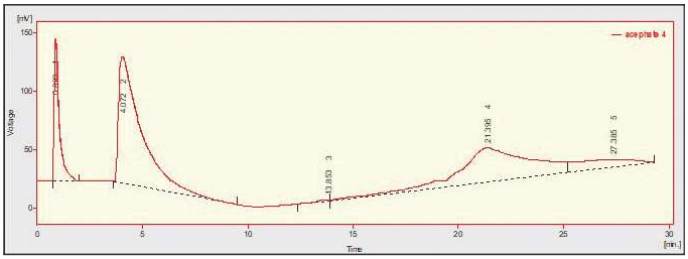

Figure 5. Extract Sample From LLE Using Hexane:Acetone, 1:1.

dimethyl phosphoramidothioate), which is more toxic than Acephate. Methamidophos is the major metabolite of Acephate. When heated to decomposition, it gives off toxic fumes of various oxides of phosphorous, sulphur and nitrogen.[14]

This research paper presents a comprehensive study on the extraction and analysis of Acephate, an organophosphate insecticide, from soil and water samples. The extraction methods employed in this study include Salting Out Liquid-Liquid Extraction (SOLLE) and Liquid-Liquid Extraction (LLE). Qualitative and quantitative analyses of Acephate were conducted using Thin Layer Chromatography (TLC), UV-Visible Spectroscopy and Gas Chromatography (GC).

Methodology:

2.1 Sample preparation : Preparation of standard solution : 10 mg standard Acephate was weighted, for working standard and transferred into 10ml volumetric flask and add about 5ml methanol and sonicated for 5min. Further fill up the remaining 5ml upto the mark with the same methanol. Further dilutions of 100ppm, 200ppm, 300ppm, 400ppm, 500ppm were made.

- 10ml of 1000ppm + 10ml methanol = 500ppm
- 1ml of 500ppm + 4ml methanol = 100ppm
- 2ml of 500ppm + 3ml methanol = 200ppm
- 3ml of 500ppm + 2ml methanol = 300ppm
- 4ml of 500ppm + 1ml methanol = 400ppm

Preparation of samples: In the experiment, a 5mg quantity of Acephate 75% SP, Acetox, was measured and placed into a 25ml Tarson tube. To facilitate mixing, 5ml of tap water was added to the tube along with 15gm of soil. The soil sample was then allowed to dry under the sun. Additionally, another 5mg of Acephate 75% SP, Acetox, powder was taken and placed into a separate 25ml Tarson tube. This time, 10ml of ground water was added to the tube. To ensure thorough mixing, the solution in the tube was vortexed for 10 minutes and subsequently subjected to sonication for 30 minutes.

2.2 Sample analysis: Method of extraction:

1. Extraction of Acephate from soil using acetonitrile
2. Extraction of Acephate from water using SOLLE (salting out liquid liquid extraction).
3. Extraction of Acephate from water using LLE.

The use of SOLLE proves to be a good method of extraction of Acephate, and polar compounds, in general. The classical method

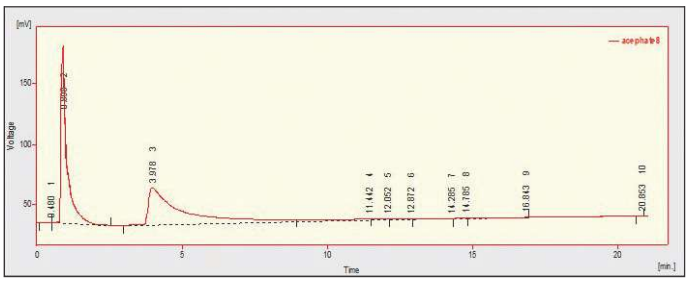

Figure 6. Extract From LLE Using Hexane (2).

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Table 1. Various solvent systems were used to find out the best solvent system:

Sl. No	Solvent system	Ratio
1	Chloroform:methanol	8:2
2	Chloroform:methanol	9:1
3	Hexane:acetone	8:2
4	Hexane:acetone	7:3
5	Hexane:acetone	6:4
6	Chloroform:acetone	7:3
7	Hexane:methanol	8:2
8	Hexane:methanol	7:3
9	Hexane:methanol	6:4
10	Hexane:methanol	5:5
11	Chloroform:ethylacetate	7:3
12	Hexane:acetonitrile	9:1
13	Hexane:acetonitrile	7:3
14	Dioxane:ethanol	8:2
15	Dioxane:ethanol:ammonia	9:1:4drops
16	Ethyl acetate:acetic acid	8:2
17	Ethyl acetate:acetic acid:ammonia	8:2:4drops
18	Dioxane:acetone	7:3

of extraction using LLE is not possible for polar compounds like Acephate. For the extraction of polar compounds, like Acephate, the general method of extraction follows the Solid Phase Extraction (SPE). It is a good extraction technique, but using SOLLE, the extraction procedure is more cost effective. It is an alternative method of extraction of polar compounds [16]. The various steps involved in SPE such as preconditioning (which involves the conditioning of the sorbent to make it compatible with the sample), loading samples, washing, elution, which is time consuming. SOLLE method is simple and less time consuming.

2.3 Extraction procedure: I. Extraction of Acephate from soil: 15g soil sample which was previously spiked with Acephate was taken to a china dish and the dried at room temperature. The soil sample was taken into a 25ml tarson tube and then 10ml of acetonitrile was added, vortex for 10min. It was then filtered, passed through sodium sulphate and then transferred into centrifuge tubes.

II. Extraction of Acephate from water sample by SOLLE: 10ml tap water is taken into 25ml tarson tube and mixed with 5mg of Acephate powder and then vortex for 10min., followed by sonication for 30min. The water sample was taken into a separating funnel and added 5ml acetonitrile and was shaken properly with hand. The solution was then allowed to settle and then saturated sugar (glucose) 5gm was added and allowed to stand still. The two miscible liquid begin to separate. The upper layer, i.e. acetonitrile layer (3.5ml) was then taken out and passed through 1g sodium sulphate.[10]

III. Extraction of Acephate from water using LLE: a. Using hexane- 5ml water sample spiked with acephate (500ppm) was taken into a separating funnel. 5ml of hexane was added to the separating funnel and gently shake for 2min. Allow the mixture to rest and collect the organic layer. This procedure was done twice; the organic layers were combined and passed through sodium sulphate. The organic layer was then concentrated by allowing it to evaporate on a water bath upto 5ml.

Table 2. Results of various solvent system after applying spray reagents.

Sl. No	Solvent system	Solvent run (a)	Solute run (b)	Under UV light (366nm)	Rf value (b/a)	Spray (Para nitro benzyl pyridine, tetra ethylene pentamine)
1.	Chloroform, methanol (8:2)	7cm	6cm	white flourescence spots	0.85	Bluish purple spots
2.	Chloroform, methanol (9:1)	5.5cm	3.8cm	white flourescence spots	0.69	Bluish purple spots
3.	Hexane, acetone, 8:2	7cm	0	white flourescence spots	0	No
4.	Hexane, acetone, 7:3	6.3cm	2.4cm	white flourescence spots	0.38	Bluish purple spots
5.	Hexane, acetone 6.4	5.5cm	3.1cm	white flourescence spots	0.56	Bluish purple spots
6.	Chloroform, acetone 7:3	5.8cm	Tailing	white fluorescence	0	No
7.	Hexane, methanol 8:2	6cm	0	No white flourescence	0	No
8.	Hexane, methanol 7:3	7cm	Tailing	white flourescence	0	No
9.	Hexane, methanol 6:4	4.3cm	1.8cm	white flourescence spots	0.41	Bluish purple spots
10.	Hexane, methanol 5:5	3.5cm	1.5cm	white flourescence spots	0.42	Bluish purple spots
11.	Chloroform, ethylacetate 7:3	5.7cm	1.4cm	white flourescence spots	0.24	Bluish purple spots
12.	Hexane, acetonitrile 9:1	6cm	0	No white fluorescence	0	No
13.	Hexane, acetonitrile 7:3	5.5cm	Tailing	white fluorescence	Tailing	No
14.	Dioxane, ethanol 8:2	6.3cm	6.3cm	white fluorescence	1	No
15.	Dioxane, ethanol, ammonia 9:1:4 drops	4.8cm	3.5cm	white flourescence spots	0.72	Bluish purple spots
16.	Ethyl acetate, a cetic acid 8:2	4.2cm	3.3cm	white flourescence spots	0.78	Bluish purple spots
17.	Ethyl acetate, acetic acid, ammonia 8:2:4drops	4.9cm	3.3cm	white flourescence spots	0.67	Bluish purple spots
18.	Dioxane, acetone 7:3	4.5cm	3.8cm	white flourescence spots	0.84	Bluish purple spots

b. Using hexane-acetone (1:1) : 5ml water sample spiked with acephate (500ppm) was taken into a separating funnel. 5ml of hexane-acetone (1:1) was added to the separating funnel and gently shake for 2min. Allow the mixture to rest and collect the organic layer. This procedure was done twice; the organic layers were combined and passed through sodium sulphate. The organic layer was then concentrated by allowing it to evaporate on a water bath upto 5ml.

1.4 Thin layer chromatography: 500ppm solution of the standard Acephate was prepared. 5µl of the standard was spotted, i.e. 2.5µg, along with 5µl of Acephate extract from soil. The spots were developed using Para nitro benzyl pyridine and tetra ethylene pentamine.

Preparation of spraying reagent: In the experiment, a solution of 2% para nitro benzyl pyridine was prepared by dissolving 1mg of

Table 3. Qualitative analysis of extracted sample using uv-visible spectrophotometry.

S.no.	Sample	Lambda max	Absorption
1.	Standard (acephate)	271	0.809
2.	Extract sample from water: SOLLE	270	3.043
3.	Extract sample from water: LLE (hexane:acetone, 1:1)	271	1.504
4.	Extract sample from water, lle using hexane:	269	0.766

Table 4. Known standards.

Sl. no.	Concentration (in ppm)	Absorption
1.	100	0.9611
2.	200	1.3961
3.	300	1.9002
4.	400	2.361
5.	500	2.8381

Table 5. Samples: extracted from water extracts (SOLLE and LLE)

Sl. no	Concentration (in ppm)	Absorption
1. Hexane: acetone, LLE	258.837	1.692
2. Hexane, LLE	140.945	1.142
3. SOLLE	473.594	2.7134

para nitro benzyl pyridine in 50ml of acetone. Similarly, a solution of 10% tetra ethylene pentamine was prepared by dissolving 1mg of tetra ethylene pentamine in 10ml of acetone. The plates were then sprayed with the 2% para nitro benzyl pyridine solution and placed in an oven for 5 minutes at a temperature of 1000°C. Afterward, the plates were sprayed again, but this time with the 10% tetra ethylene pentamine solution. As a result, blue-colored spots were observed on the plates. The Rf (retention factor) value was calculated as part of the analysis.

2.5 UV/visible spectrophotometer - The experiment began by switching on the instrument and allowing the lamps to warm up for 20 minutes. Once ready, the SCANALYSE Software was opened and the wavelength range was set between 200nm and 400nm. The range scan option was chosen from the toolbar. To establish the baseline, both cuvettes were filled with the reference solution (Methanol) and placed in the sample holder. After setting up the baseline, each of the prepared standards and extracts were individually scanned and their respective spectra were recorded. To prepare the standard Acephate, 10ml of a 1000ppm solution was initially created. Subsequent dilutions were made as follows:

- - 10ml of 1000ppm + 10ml methanol = 500ppm
- - 1ml of 500ppm + 4ml methanol = 100ppm
- - 2ml of 500ppm + 3ml methanol = 200ppm
- - 3ml of 500ppm + 2ml methanol = 300ppm
- - 4ml of 500ppm + 1ml methanol = 400ppm

Next, a calibration curve was plotted using the prepared working standards of different concentrations to determine linearity. This step was based on Beer Lambert's law, which states that concentration is directly proportional to absorbance. Finally, the extracts from soil and water were scanned to quantify the target substance.

2.6 Gas chromatography: The instrument was switched on and then programming of various temperature flow rate was done.

Programming:

Column – DB 624, 30mm, 3µm, 0.32 id

Injector temperature – 2500C

Flow rate N2 – 50cm/min

Pressure – 5.57 psi

Temperature – ramped to 2500C

Oven temperature – start at 1000C, raise it to 1800C at 250C/min, hold for 3min raise it to 2000C at 40C/min, hold for 1min and up to 2500C at 100C/min.

Detector – 2700C ECD

1µl standard solution of Acephate of 800ppm was injected into the injection port. After the standard was run, 1 µl of the extracts (from soil and water) were injected into the injection port under the same condition. Retention time of the standard and the extracts were noted down, also the peak area, for quantitation.

Result and discussion:

Acephate, a water soluble pesticide was spiked in water (500ppm). It was extracted using SOLLE (sugaring out liquid liquid extraction) and LLE (liquid liquid extraction).

3.1 Thin layer chromatography: Selection of suitable solvent system for Acephate. The existing normal solvent system, mentioned in DFS manual, hexane and acetone, does not give good result for acephate.

Spraying reagent: 2% Para nitro benzyl pyridine and 10% Tetra ethylene pentamine. After the TLC was run, the plates were sprayed with para nitro benzyl pyridine. The plates were kept in oven for 5min at 1000 C. The plates were removed and sprayed again with tetra ethylene pentamine. Blue color spots were observed, indicates thiophosphorous group. Rf value was calculated after spraying with reagent.

Various solvent systems were used and found chloroform, methanol (9:1) and dioxane, methanol, ammonia (9:1:4µl) to be the most suitable solvents for acephate as the spots were resolved, prominent and round. (See Table.1 and 2). The spray reagent used was stable upto 5 hrs, after which the color of the spots on the TLC plate disappeared.

3.2 UV-vis spectrophotometer: The result of qualitative analysis of extracted samples are given in (Table .3).

Linearity graph: (see Figure: 2).

3.3 Quantitative analysis: The calibration was made from 100ppm to 500ppm.

The lamda max of the standard and the extracted samples were

Table 6. Data interpretation by GLC.

Sl. no.	Name	Retention time (min)	Peak area	Concentraction (ppm)
1.	Acephate standard	4.100	13716.153	800.00
2.	Extraction by SOLLE method	4.072	8184.026	477.33
3.	Extraction by LLE (Hexane:Acetone, 1:1)	4.048	4149.373	242.01
4.	Extraction by LLE using hexane	3.978	2558.796	149.24

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found to be similar i. e. 270nm (see Table. 3) . Quantitative analysis was performed for the extracts from water and found to have 94.71% by SOLLE, 54.71% by LLE (hexane:acetone 1:1), 28.18% by LLE(hexane). Thus, the use of SOLLE technique for the extraction of Acephate was found to be better than LLE method using hexane or hexane:acetone (1:1). (See Table. 4 and 5).

3.5 Gas chromatography:

Programming: Column – DB 624, 30mm, 3µm, 0.32 id

Injector temperature – 2500C

Flow rate N2 – 50cm/min

Pressure – 5.57 psi

Temperature – ramped to 2500C

Oven temperature – start at 1000C, raise it to 1800C at 250C/min, hold for 3min raised it to 2000C at 40C/min, hold for 1min and up to 2500C at 100C/min.

Detector – 2700C ECD

Amount of injection – 1µl

I. Standard acephate (See Figure:3).

II. Extract Sample from Solle (See Figure:4).

III. Extract sample from lle using Hexane: Acetone, 1:1 (See Figure:5).

IV. Extract from lle using Hexane (2) (See Figure.6).

Calculation of concentration of Acephate in the extracts was done by comparing peak area.(See Table. 6).

Area of standard/concentration = area of the extract/ concentration of the extract i.e. concentration of Acephate in the extract = area of the extract x concentration of the standard/area of the standard 500ppm of acephate was spiked in each of the following extract procedure.

Percentage recovery of samples:

1. Extract using SOLLE – 95.46%.
2. Extract using LLE (hexane:acetone, 1:1) – 48.40%.
3. Extract using LLE (hexane) – 29.84%.

Thus, the use of SOLLE technique for the extraction of Acephate was found to be better than LLE method using hexane or hexane:acetone (1:1).

Conclusion:

The solvent system mentioned in Directorate of Forensic Science manual, Forensic Toxicology i.e, Hexane:Acetone for pesticides did not yield good result for Acephate pesticide, therefore, various other solvent systems for TLC were studied and the suitable solvent system found were Chloroform:Methanol (9:1) and Dioxane:Methanol:Ammonia(9:1:0.3). These solvent systems were comparatively better, because, the Rf values, shape of the spots. These solvent systems newly developed are not mentioned in the literature. The UV-Vis Spectrophotometric analysis is based on the experimental results and is not mentioned in the literature. The lamda max of the standard Acephate was found to be 271nm. The extraction of water soluble Acephate insecticide was done using LLE and SOLLE methods. The recovery percentage of the extracts from water using LLE was 48.40%, and 95.46% using SOLLE. Normally, LLE method of extraction is used for extraction of Acephate, so for better recovery percentage of Acephate, SOLLE method is suggested. The recovery percentage was done using Gas Liquid Chromatography. The percentage recovery was also studied using alternative technique, UV-Vis spectrophotometer and found to have 94.71% by SOLLE, 54.71% by LLE (hexane:acetone 1:1). So the percentage recovery of Acephate has been successfully done by using Gas Liquid Chromatography and UV-Vis spectrophotometry.

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