Study of Heavy Metals from Seed Oil of Nicotiana Tabacum Plants of Solanaceae Family from Arid Zone

Neelam Sonal

Department of Chemistry, Jai Narain Vyas University, Jodhpur -342001 (Rajasthan) India
Email: *neelam202530[at]gmail.com*

Abstract: ***An important source of oils with pharmacological, industrial, and nutritional benefits is plant seeds. An oil is appropriate for a certain use depends on its properties and fatty acid composition. Nicotine is an alkaloid found in tobacco leaves (Nicotiana tabacum). The chemical that causes smoking to become such an addictive habit is nicotine, which is both well-known and notorious. Nicotiana tabacum belongs to the family Solanaceae. The oil content, Protein content and moisture (by solvent extraction method in petroleum ether) of Nicotiana tabacum were found 37.5%, 35.5%, and 5.6%, respectively. The Soxhlet equipment was used to extract the oil, and AAS (Atomic Absorption Spectroscopy) revealed the presence of heavy metals such as Fe, Zn, Cu, Cd, Cr, Ni, Co, and Pb in Nicotiana tabacum seed oil. In Nicotiana tabacum seed, the Fatty acids were found, specially, Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid, Linoleic acid (C18:2) and Linolenic acid (C18:3) as 9.60%, 8.5%, 2.5%,13.5%, 72.03%, and 27.97%, respectively. Iodine value (136.8 g I2/100g), Saponification value (186.6 mg/g KOH), un-saponification value (1.42% w/w), and Peroxide value (meq. /kg) (1.92) are the physicochemical characteristics of Nicotiana tabacum. The Physico-chemical characteristics of the seed oil of Nicotiana tabacum were determined using AOCS methods.***

Keywords: Fatty acids, Nicotiana tabacum, Iodine value, AAS, alkaloid, Heavy metals

1. Introduction

The Solanaceae family of shrubs primarily consists of annuals. A key component of the indigenous medicine pathway is the genus Nicotiana tabacum. Scattered across temperate and tropical regions, the Solanaceae family, also referred to as the nightshade family, is a dicotyledonous flowering plant family that has roughly 98 genera and 2700 species [1]. Nicotiana tabacum belongs to the Solanaceae family. It is one of the most popular medicinal crops that are grown commercially as a dry land crop in India during the late Kharif season. Oilseed crops hold great significance or worth in the agricultural sector. By-products of Nicotiana tabacum processing, especially seeds, are stores of health-promoting macromolecules like vitamins (α-tocopherol), carotenoids (lycopene), proteins (bioactive peptides), polysaccharides (pectin), and phytochemicals (flavonoids) [2]. Nicotiana tabacum is a beneficial ingredient for the creation of functional meals because of its health-promoting bioactivities [3]. Numerous studies have been conducted on the nutritional and phytochemical characterizations of Solanaceae by-products during processing paste [4]. Fruits and vegetables are two of the most popular foods consumed worldwide [5]. Similar to edible portions, fruit and vegetable leftovers are rich in nutrients and phytochemicals [6]. Heavy metals are found in Nicotiana tabacum seed oil samples using a technique called AAS (Atomic Absorption Spectroscopy). This was one of the most popular instrumental methods for metal and certain metalloid analysis. Metals in food samples are also commonly identified using these techniques. Solid materials must be dissolved before analysis using the most popular commercial apparatus of this type, which was designed for liquid samples. Atomic absorption spectroscopy can be used to identify metals in liquid samples. Metals that can be analysed include Fe, Cu, Al, Pb, Ca, Zn, Cd, and others. When heavy metal levels of the medicinal plant tabaco were measured, it was found that the plant has considerable concentrations of these metals in its seeds [7-9]. The project is to increase public awareness of the detrimental health consequences of high heavy metal concentrations as well as the proper use and harvesting of medicinal plants. They are extremely dangerous, even at very low concentrations [10–14]. Despite being essential nutrients, trace heavy metals such as Fe, Cu, Zn, Cu, Mn, and Ni are toxic and harmful when their quantities exceed recommended levels [15–17]. Lead, cadmium, and other heavy metals are superfluous. Nicotiana tabacum plant is an annual plant that grows 1 to 3 m high and is sticky hairy on all parts (Table 1, Fig. 1, 2).

Table 1: A brief detail regarding Nicotiana tabacum plant

S. No.	Botanical name	Nicotiana tabacum
1	Common name	Tabacoo
2	kingdom	Plantae (plants)
3	Division	Magnoliophyta (flowering plant)
4	domain	Eukaryota
5	class	Magnoliopsida (dicotyledons)
6	subclass	Asteridae
7	order	Solanales
8	genus	Nicotiana
9	species	N. tabacum
10	Part used	Leaf, seeds, fruit
11	Medicinal properties	relieve pains and inflammatory diseases and stomach problems
12	Chemistry	Palmitic, oleic linoleic acids, Trilinolein and palmitodilinolein
13	Regional habitat	Jodhpur, Pali, Barmer, Naguar etc.
14	Medicinal use	addiction to or dependence on smoking cigarettes.

The stems are thick and not very branched. The leaves can be over 50 cm long with the blades ovate to elliptical, or obovate, pointed towards the front and, at the base, run down the stem or are sessile, encompassing the stem. The scented inflorescences are multi-branched panicles. The flower stalks are 5 to 15 mm long. The calyx is 12 to 18 mm and is covered with uneven 4 to 8 mm narrow pointed calyx lobes shorter than the calyx tube. The crown is plate-shaped, the coronet is white, pink or red, the corolla tube greenish-cream, pink or red. The corolla tube has a total length of 3.5 to 4.5 cm and is 3 to 5 mm wide in the lower part and widens to 7 to 12 mm in the upper part. The coronet is lobed or pentagonal. The stamens are designed unevenly and start below the center of the corolla tube. The anthers of the four longer stamens are close to the opening of the corolla tube or are slightly above it. The fifth stamen is significantly shorter than both longer pairs. The stamens have a length of 2.5 to 3.5 cm, significantly longer than the anthers, and are hairy at the base. The fruit is a 1.5 to 2 cm long capsule that is narrowly elliptical to egg-shaped.

2. Materials and Methods

2.1 Materials

The seeds were harvested from the dry and semiarid regions of western Rajasthan, India, once they had fully grown (Fig. 3). For the studies, the complete seed was used; it was immediately evaluated after being freeze-dried and broken into a powder using a mortar.

2.2 Apparatus

In Figure 4, every significant component of the Soxhlet device is displayed. These components include the following: expansion adapter, condenser, extraction flask, distillation channel, thimble, plant seed flour, syphon top, syphon exit, stirring bar and cooling water inlet and outlet.

Figure 1: Plant of Nicotiana tabacum, an arid zone plant belonging to the Solanaceae family

2.3 Sampling

For preparing samples in order to study heavy metal content accumulated in seeds. Fruits samples of four species from Solanaceae family were collected from nearby Jodhpur. Seeds from these selected fruits were extracted and wash with water several time and dried properly in sun light for 4-5 days. After 4-5 days drying, now the seeds were ready for extraction of oils. Now 500gram seeds were weighed for each four plant species. These samples were further crushed with the help of motor and pastel.

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Figure 2: Flowers of Nicotiana tabacum, an arid zone plant belonging to the Solanaceae family

2.4 Method

The experimental work is shown in the **Figure 4.** The experiment starts with the determination of oil content and then the oil is characterized. After that, the extracted oil is refined and then be characterize again. Lastly, the refined oil is modified using sulfation method

Figure 3: Seeds of Nicotiana tabacum, an arid zone plant belonging to the Solanaceae family.

2.4.1 Oil Extraction: Oil was extracted from **Nicotiana tabacum seeds** Fig. 3a, and b) using moderate petroleum ether at 40–60 degrees Celsius, following the Soxhlet extraction method. To completely extract the solvent under vacuum, a revolving evaporator was employed. The seed and seed oil analytical values were determined using the

American Oil Chemist Society's (AOCS) standard techniques. We also performed TLC and alkaline picrate assays to check for any unusual fatty acids. The oil from crushed seeds from desired plant species were extracted by repeated washing (known as percolation) with petroleum ether (boiling range between 40-60^{O}C) and using the Soxhlet's procedure [5].

3. Results and Discussion

Specific gravity, melting point, saponification value, percentage of unsaponifiable value, acidic value, and FA content are among the physicochemical characteristics of **Nicotiana tabacum** that vary. Table 3,4 and 5 lists the AOC's technique for obtaining the physicochemical characteristics and FTIR of Nicotiana tabacum (Fig. 6) seed oil, respectively.

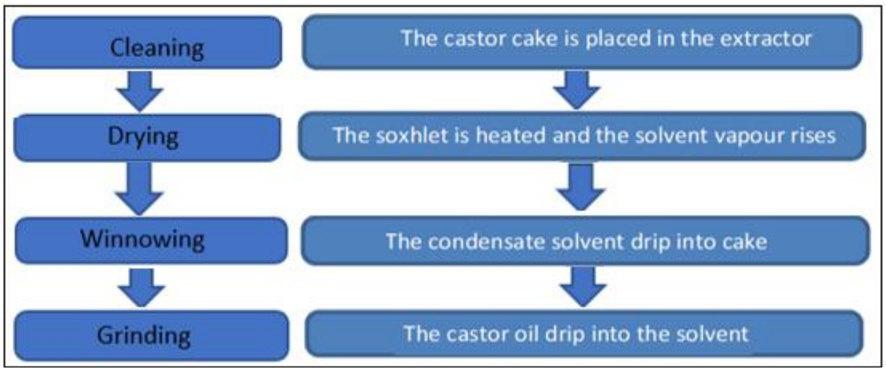

Figure 4: Flow chart of seeds Sample processing

3.1 Physical Properties of Oils and Fats

3.1.1 Acid value

Accurate monitoring of acid value (AV) is critical for edible oil quality control, yet traditional chemometric methods often face limitations in handling complex spectral data [18].

Acid value (AV) is the amount of potassium hydroxide in milligrams needed to neutralise one gramme of fat's FFA. Regardless of molecular weight, it is, ironically, about twice as high as FFA% when expressed on an oleic-acid basis (i.e., fat, oil, or chemical substance). Simply said, the number of carboxylic acid groups in a fatty acid can also be used to calculate the acid number. Using phenolphthalein as an indicator, a solution of fat or oil with a given weight (W) in an organic solvent is titrated with a potassium hydroxide solution of known strength in order to calculate the acid number of the substance. Therefore, the amount of acid in a sample of biodiesel, fat, or oil can be measured using the acid number. Since unrefined oil has a high acid value, the acid number also reflects the quality of the oil. The following formula was used to determine the acid value:

$$\textbf{Acid value} = \frac{\mathbf{V \times N}}{\mathbf{W}} \times \mathbf{56.11}$$

Whereas: V = volume of KOH solution consumed, N = Normality of KOH solution, W = weight of the sample in gram.

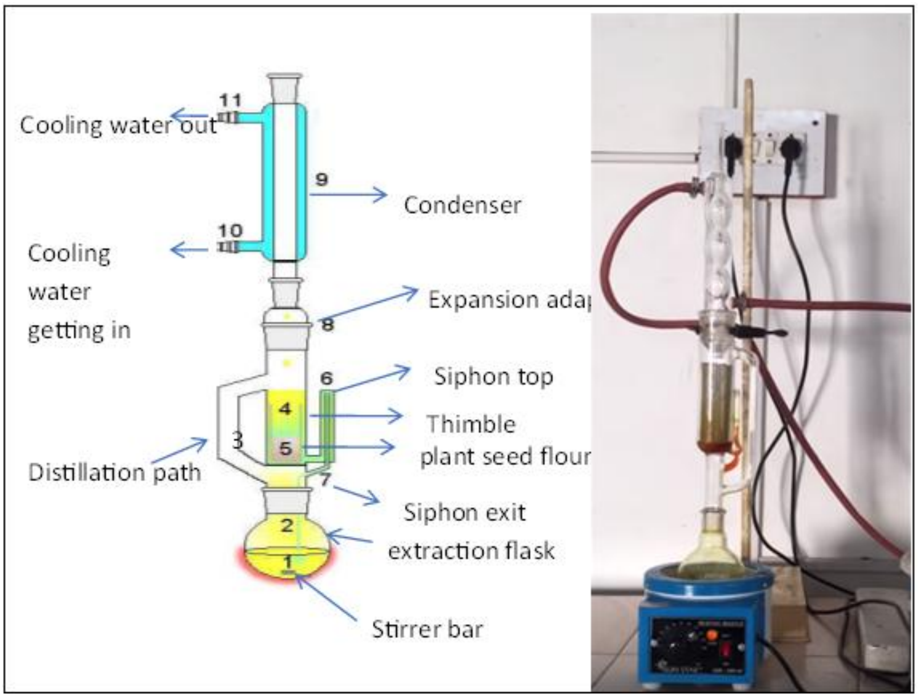

Figure 5: A typical Soxhlet apparatus and its set up for extraction of seed oil

3.1.2 Iodine value: A fat or oil's iodine value is the "weight of iodine needed to absorb 100 grammes of fat or oil." The following formula was used to determine the iodine value of seed oil:

$$\textbf{Iodine value} = \frac{(\textbf{Blank exp}) \times \textbf{0.1269}}{\textbf{Weight of sample in gram}}$$

3.1.3 Saponification value: Saponification value, the average molecular weight of fatty acids, is a crucial parameter for detecting adulteration of edible oils [19]. A fat or oil's saponification value is the "number of milligrammes of potassium hydroxide required for saponification of 1 gramme of fat or oil". A measure of the fatty acid constituents' molecular weight or size in relation to their chain lengths is the saponification value. For example, fatty acids with a high molecular mass are indicated by a saponification value of about 195, while fatty acids with a low molecular mass are indicated by a saponification value of around 300. When manufacturing soap, oils with a high saponification value are thought to be highly helpful. The following formula was used to determine the saponification value of seed oil:

$$\textbf{Sponification value} = \frac{(\textbf{A} - \textbf{B}) \times \textbf{56.11}}{\textbf{Weight of sample in gram}}$$

Whereas: A = volume of H_2SO_4 solution used in the blank titration (ml), B = Volume of H_2SO_4 solution used in the sample titration (ml), N = Normality of H_2SO_4 solution

Table 2: Physico-chemical properties of the seeds and oil of Nicotiana tabacum

S. No.	Parameters	Obtained value
1	Peroxide value (meq. /kg)	1.92
2	Iodine value (g/100g)	136.8
3	Saponification value (mg of KOH/g)	186.6
4	Un-saponified matter (%)	1.42

3.1.4 Peroxide value

In most cases, oxidation of fats and oils results in the production of hydrogen peroxide. Oxidative rancidity is the process by which fats and oils are oxidised by air. "Ketonic rancidity" is the term used to describe the oxidation of oils and fats caused by microorganisms. varied oils require varied amounts of time to produce rancidity and varying degrees of peroxide production. Therefore, the peroxide value is just a measurement of the amount of peroxide present in fats and oils. The following formula was used to determine the seed oil's peroxide value:

$$\textbf{Peroxidevalue}(\textbf{mEq/kg}) = \frac{\textbf{S} \times \textbf{N} \times \textbf{100}}{\textbf{Weight of sample in gram}}$$

Whereas A = Volume of $Na_2S_2O_3$ solution in the blank titration (ml), S = Normality of $Na_2S_2O_3$ solution

3.1.5 Percentage of Unsaponifiable Matter

There is a trace quantity of additional stuff in fats and oils. These materials are referred to as unsaponifiable matter because they do not alter with saponification. The percentage of unsaponifiable matter (USM) was calculated by first saponifying the oil sample with 0.5M alcoholic potassium hydroxide and then extracting it with diethyl ether in a glassware separating funnel to separate the unsaponifiable matter. As a result, the unsaponifiable material that was obtained was crude and washed with water before the organic solvent evaporated. After being dried in an oven at 105°C, the unsaponifiable material was weighed and the percentage was determined using the formula below:

$$\textbf{\% USM} = \frac{\textbf{mass of the extracted USM in g (P)}}{\textbf{mass of the oil sample saponified in g (M)}} \textbf{ x 100}$$

3.2 Plant seed oil content determination

As previously mentioned, the Soxhlet apparatus was used in the preceding section to extract the seed oil. To extract oils, 10 grammes of seed flour were combined with a solvent and placed within a Soxhlet apparatus. The solution was concentrated and filtered via filter paper following solvent extraction. With the use of a rotary evaporator, the solvent was removed, and the resulting oil was then dried in an oven set at 105°C for more than 20 minutes in order to eliminate any solvent residue. The following formula was used to quantify the mass of oil and determine its percentage:

$$\textbf{\% of Oil} = \frac{\textbf{Mass of oil (P)}}{\textbf{Mass of seed flour (M)}} \textbf{ x 100}$$

3.2.1 Protein content

Protein is a crucial component of seed oils and is necessary for human growth. Therefore, to determine the benefits of seed oils for consumption, the protein content of the oil must be determined. The Kjeldahl method was employed to ascertain the protein content of seed oils. The following formula was used to determine the percentage of protein and nitrogen in seed oil:

$$\% \text{ N} = \frac{(\text{N}_1\text{V}_1 - \text{N}_2\text{V}_2) \times 1.4007}{\text{mass of the oil sample (g)}}$$

Whereas:N_1 = Normality of HCl solution, N_2 = Normality of NaOH solution,V_1 = Volume of HCl solution,V_2 = Volume of NaOH solution, % Protein = % N × 6.25

Table 3: Different parameters (%) of seed oil in Nicotiana tabacum

S. No	Parameters	Obtained value
1	Oil content (%w/w)	37.5
2	Protein content (%w/w)	35.3
3	Moisture (%w/w)	5.6
4	Acid Value	3.75
5	Melting point	19º67
6	Refractive index (at 40ºC)	1.4598

3.4 Heavy Metal Analysis

Large-scale technologies used in inorganic analytical labs, such as graphite furnace atomic absorption spectroscopy (GF-AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), X-ray fluorescence spectroscopy (XRF), flame atomic absorption spectroscopy (FAAS), and inductively coupled plasma-mass spectroscopy (ICP-MS), can be employed to investigate the heavy metal content in seed oil. All of these analytical methods, however, differ in terms of sensitivity, sample preparation, and analysis costs. For the determination of heavy metals in this investigation, atomic absorption spectroscopy (AAS) was selected due to its precision, excess, and ease of use in sample preparation. As

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a result, the instrumental methods are briefly described. The components of heavy metals were quantitatively estimated using AAS method. The metal content of the sample is directly correlated with the absorbance in the atomic absorption spectrometer. Therefore, the metal content of plant seed oils is measured using an atomic absorption spectrophotometer. The wavelength dial was adjusted according to the kind of metal that was discovered in the sample and needed to be analysed. Nicotiana tabacum seed oil contained unsaturated fatty acids like linoleic acid (C18:2), oleic acid (C18:1), and linolenic acid (C18:3), and Saturated fatty acids like myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) were present. Unsaturated fatty acids, especially linoleic and oleic acid, are more abundant in Nicotiana tabacum seed oil, according to GC-FID spectroscopy. Tobacco (Nicotiana tabacum L.) is a widely cultivated crop globally, recognized for its substantial economic, agricultural, and social significance [20].

Myristic acid (C14:0), palmitic acid (8.5%), and stearic acid (2.5%) were the most common saturated fatty acids, the study found. Linolenic acid (C18:3) accounted for 27.97% of the polyunsaturated fatty acids, while oleic acid (13.5%) and linoleic acid (72.03%) were the unsaturated fatty acids (Table 4)

Table 4: Fatty acid composition of seed oil of Nicotiana tabacum

S. No.	Fatty acid	% of fatty acid
1	Myristic acid (C14:0)	9.60
2	Palmitic acid (C16:0)	8.5
3	Stearic acid (C18:0	2.5
4	Oleic acid	13.5
5	Linoleic acid (C18:2)	72.03
6	Linolenic acid (C18:3)	27.97

The heavy metal analysis of tabaco seed oil reveals that although there are major levels of Fe (55.69 mg/L), Zn (26.9 mg/L), and Cr (22.45 mg/L), Cu (15.36 mg/L), Ni (4.16 mg/L), and Cd (2.67 mg/L) are present on average, while trace amounts of Co (1.16 mg/L) and Pb (1.50 mg/L) have been detected(Table 5).

Table 5: Concentration of heavy metals detected in seed oil of Nicotiana tabacum

S. No.	Metal	Concentration unit (mg/L)
1	Fe	55.69
2	Zn	26.9
3	Cu	15.36
4	Cd	2.67
5	Cr	22.45
6	Ni	4.16
7	Co	1.16
8	Pb	1.50

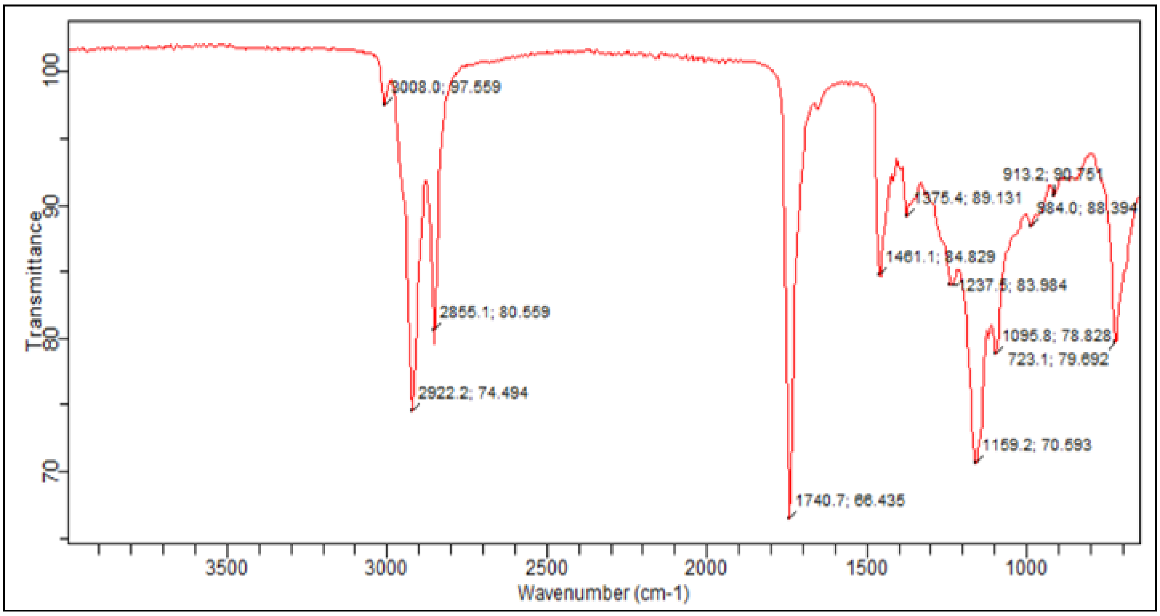

Figure 6: FTIR spectra of Nicotiana tabacum

4. Conclusions

Seed oil is a rich source of essential Omega-6 (linoleic acid C18:2) fatty acids (**Table 4).** The most significant polyunsaturated fatty acid in the human diet is linoleic acid, which protects against heart and vascular diseases. Therefore, seed oil is good for human health. Linoleic acid can be used in place of paint and varnish due to its high PUFA content. In addition to being consumed by people and used in industry, oilseed and plant component byproducts could be used as biomass in a number of applications and as animal feeding. Because larger concentrations of heavy metals in seed oils signify a high level of contamination in the area where the plants were growing, plants can be used as bio-indicators of pollution. Tabaco seed oil has high levels of the heavy metals Fe, Zn, Cr, and Cu (Table 5). On the other hand, trace amounts of Pb, Cd, and Co metals are found. The type of metal, the physicochemical properties of the soil, and the type of plant all affect how metals behave in soils and how they are absorbed by plants. To solve the investigation's purpose, seed oil is digested and analysed using atomic absorption spectroscopy (AAS).

Key findings:
- Research on the analysis of heavy metals in Nicotiana tabacum plant seed oil. An improved solanaceae family from the desert zone.
- Considering its appropriateness, biodiesel is a novel area of research to facilitate commercialization.
- There are a lot of heavy metals in the document.
- To feed everyone on the planet, the scientific community must find sustainable solutions.

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Ethical Approval: All authors approved that this study is non-human subject research, and no need for informed consent.

Consent to Participate: Not applicable

Consent to Publish: Not applicable

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