# EXTRACTION AND IDENTIFICATION OF ATROPINE FROM "LEGAL HIGH" PLANT SPECIES

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**Abstract:** Atropine from herbs has been used in traditional medicine over centuries, mainly because of its hallucinogenic properties. When administrated orally through abusing of *Datura stramonium* (DS) seeds, atropine is quickly absorbed causing dilatation of the pupil, tachycardia, hyperthermia, dizziness, nausea, extreme confusion, deliriant hallucinations and in some cases death. Knowing that *Datura stramonium* is one of the "legal high" plants with a high risk of toxicity for humans and animals, forensic analysis of such a material is of high importance. This work aimed at developing a fast and simple method for extraction and determination of the atropine content as the main alkaloid in *DS* seeds. Optimization of parameters for conventional heated solid-liquid extraction of atropine from *DS* seeds were obtained by varying the particle size (8.6 mm-1.7 mm), temperature (40°C-60 °C), and ethanol concentration (48.0-96.0 % v/v). The extracts were analyzed by Acquity UPLC HSS C18 1.8 μm column with mobile phase acetonitrile and formate buffer pH 3.6 (75:25) and flow rate of 0.4 mL/min. Quantitation was done in MRM mode using m/z 290→ 124 and 290→ 93 transitions.

**Keywords:** *Datura stramonium*, drug, atropine, extraction, MS-MS spectrometry

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## INTRODUCTION

The abuse of psychoactive substances is one of the biggest challenges faced by legal authorities in modern society. Although the abuse of synthetic drugs produced in illegal laboratories around the world is particularly widespread among the youth population, teenagers are not unfamiliar with the drugs they can find in nature, such as plant *Datura Stramonium L (D. Stramonium)*.

*D. Stramonium* also known as *Jimson weed* is an annual summer plant widely distributed and usually found in abandoned areas, along roads and in cultivated fields. The plant particularly prefers sandy soil or a calcareous loam and open sunny position, while nitrogen-enriched soil, such as fertilized soils, contributes to a richer alkaloid content (Chopra, 2006). *D. Stramonium* is a cylindrical branched plant that can reach a height of 0.5 to 1.5 m. During the summer the plant is veiled with the solitary, white, trumpet-shaped flowers that open at night, emitting a pleasant fragrance. In the period of blooming, each flower is replaced by a dry, pointed and oval-shaped fruit. The immature fruit is green coloured and covered with soft spines, and when riped it becomes brown coloured, covered with sharp spines and splits into four chambers which contain small, black seeds (Figure 1). Up to 200 seeds can be found in capsules (Gaire & Subedi, 2013; Krenzelok, 2010). *D. Stramonium* is a well-known plant used in Eastern medicine to treat ulcers, wounds, inflammation, fever, asthma and sinus infections, rheumatism, etc. (Kuete, 2014). However, the frequent recreational abuse of the plant mostly among adolescents and young adults has resulted in toxic syndromes.

Figure 1. *Datura Stramonium L. a) flower (left), b) the mature fruit with seeds (right)*

*D. stramonium* contains saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides. Although all parts of the *D. stramonium* plant contain tropane alkaloids ((hyoscine (scopolamine), atropine (dl-hyoscyamine)), which are classified as deliriants, the ripe seeds contain the highest concentration (Kuete, 2014; Soni et al., 2012). One seed contains about 0.1 mg of atropine (Trancă, Szabo, & Cociș, 2017) and the initial symptoms can appear several hours after oral ingestion of the seeds (Artal, 2015). It can significantly affect heart function and cause tachycardia, tachypnea, hypertension, urinary retention, but also several neurological symptoms such as agitation, delirium, dilated pupils, disorientation, hallucinations, seizures, photophobia, and coma (Kohnen-Johannsen & Kayser, 2019; Stellpflug, Cole, Isaacson, Lintner & Bilden, 2012). Atropine is a competitive antagonist of muscarinic receptors, so it can cause the effects which the psychoactive substance users seek to achieve, such as euphoria and hallucinations. In large doses, tropane alkaloids are toxic, and death is often caused by the ingestion of large amounts of seeds of the plant *D. Stramonium* (Grynkiewicz & Gadzikowska, 2008).

Bearing in mind the fact that plants from *Datura* genus contain significant amounts of tropane alkaloids to which particular attention should be given due to toxic effects on human and animal health, extraction and analysis of alkaloids from such plant material are highly important for forensic science.

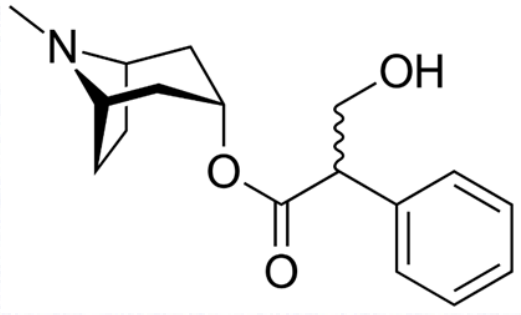


Figure 2. *Chemical structure of atropine*

The extraction of tropane alkaloids from *D. Stramonium*, the type of plant that belongs to the family *Solanaceae* was carried out using different techniques including supercritical fluid extraction (Brachet et al., 1999), ultrasonic extraction (Djilani & Legseir, 2005), solid-liquid membrane extraction (Noori & al-Hemiri, 2009), microwave-assisted extraction (Ciechomska et al., 2016), column extraction with ultrasonic bath (Abbaspour, Khadiv Parsi, Khalighi-Sigaroodi & Ghaffarzadegan, 2016), and column liquid-liquid extraction (Śramska et. al, 2017). For the analysis of atropine and scopolamine in the plant extracts the gas chromatography-mass spectrometry (Ciechomska et al., 2019), liquid chromatography-mass spectrometry (Jakabová et al., 2012), high-performance liquid chromatography coupled ether with UV-Vis spectrophotometer or diode-array detector (Sawabe et al., 2011) or with mass spectrometry (Steenkamp, Harding, Van Heerden & Van Wyk, 2004), and capillary electrophoresis-mass spectrometry (Gao, Tian & Wang, 2005) were used.

Although there are many techniques used for extraction of tropane alkaloids, their isolation from the complex plant matrix is challenging and often time-consuming task, not typically preferred in forensic chemistry. So, this paper aims at presenting the relatively fast and simple method for extraction and determination of the atropine content as the main alkaloid found in *D. Stramonium* seeds.

## MATERIALS AND METHODS

The ripe fruits of *D. Stramonium* were collected during the period of blooming from different localities of central Serbia. The fruits were dried immediately after harvesting at room temperature in a well-ventilated space and then stored in a dark place in a paper bag until use. Before extraction, the seeds were taken from the dried capsules and then ground in an electrical mill (*Bosch electric grinder Germany*, power 180W). For optimization study, the plant seeds (~2 % moisture content) were ground for the predetermined period in order to obtain the different particle sizes. The average size of the seed particles was measured by sieving, using different size meshes.

Ethanol (p.a., Zorka Pharma, Serbia) was used as a solvent for the extraction. The solvent of certain concentration (% v/v) was prepared before each experiment. For preparation of mobile phase, the LC grade solvents (Sigma Aldrich) were used. The pure atropine (≥ 99 %) was purchased from Sigma Aldrich.

## Conventional solid-liquid extraction of atropine from D. Stramonium seeds

Ground seeds of *D. Stramonium* (5 g) were suspended in a solvent (75 ml) in Erlenmeyer flask which was placed in a water-filled baker and continuously stirred (300 rpm) using a magnetic stirrer (Velp Scientifica, Italy) for 90 min at constant temperature. The temperature of the reaction suspension was monitored by a digital thermometer immersed in an Erlenmeyer flask.

To optimize the atropine extraction process, extraction was performed by changing the particle size of *D. Stramonium* seeds (dp=8.6 mm (denoted as $m_1$); dp=3.3 mm ($m_2$); dp=2.2 mm ($m_3$); dp=1.7 mm ($m_4$)), temperature (40 °C, 50 °C and 60 °C (± 1 °C)), and ethanol concentration (48.0 % v/v , 72.0 % v/v , 96.0 % v/v ). After extraction, the ethanol extracts were cooled using the ice bath and filtered through the filter paper and the obtained extracts were subjected to analysis.

## Preliminary tests of ethanolic extracts of D. Stramonium

For the preliminary test which tends to indicate the presence of atropine in the obtained extracts, an alkaloid detecting reagent (Dragendorff's reagent) was used. Dragendorff's reagent was prepared according to Ameh et al. (2010). In short, two solutions were prepared as follows: Solution A (1.7 g basic bismuth nitrate dissolved in 100 ml of water: acetic acid solution (4:1)), and Solution B (40.0 g potassium iodide was dissolved in 100 ml of water). Two solutions were then mixed to yield 100 ml of reagent (Solution A (5 ml), Solution B (5 ml), acetic acid (20 ml) and water (70 ml)). The ethanolic extracts (3 mL) obtained within each of the individual experiments were evaporated and then the solid residues were dissolved in methanol. The aliquots of extracts (1 mL) were transferred into the test tubes and treated with the prepared Dragendorff's reagent (1 mL). The development of red to orange colour indicated the presence of atropine.

## Analysis and quantification of atropine in ethanolic extracts of D. Stramonium

Ultra-performance liquid chromatography–tandem mass spectrometry (UPLC/MS/MS) method was used for the determination of atropine in ethanolic extracts of *D. Stramonium.* The obtained extracts from each individual experiment were analysed on Acquity UPLC HSS C18 1.8 μm column with mobile phase acetonitrile and formate buffer pH 3.6 (75:25) and flow rate of 0.4 mL/min. Positive ionization tandem MS detection in the multiple reaction monitoring (MRM) mode was used for quantification, using m/z 290→ 124 and 290→ 93 transitions.

# RESULTS AND DISCUSSION

The obtained extracts were firstly preliminary tested for presence of tropane alkaloids. Figure 3 shows aliquots of different ethanol extracts tested with Dragendorff's reagent.

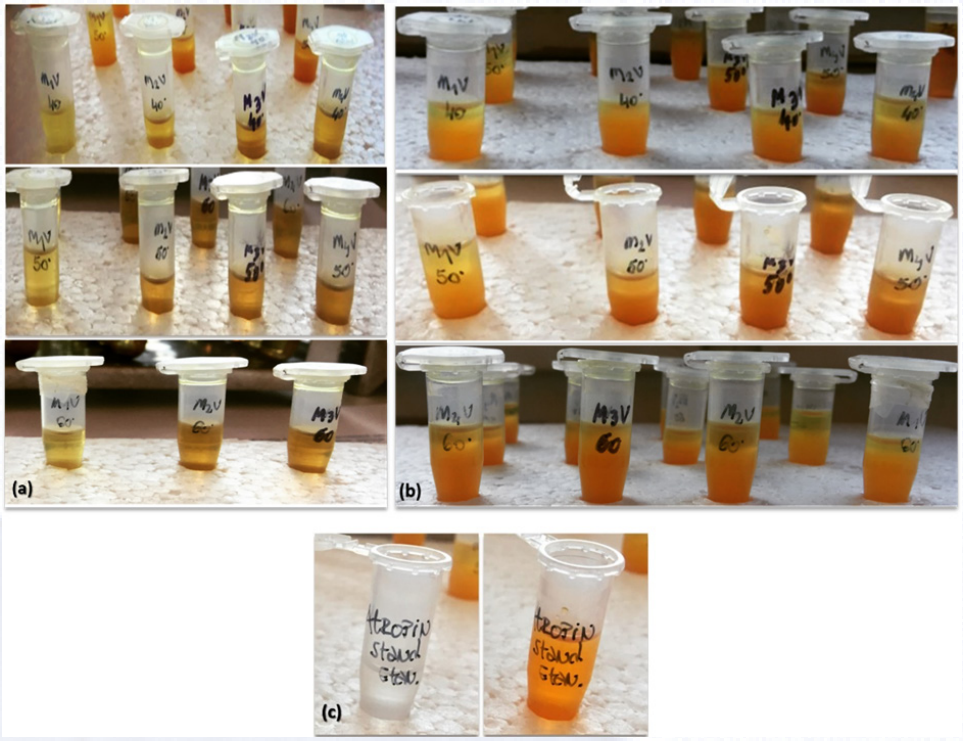


Figure 3. Extracts of D. Stramonium seed obtained at different temperatures (40 - 60 °C) and when using ground seeds of different particle size ($m_1$ - $m_4$) before (a), and after adding the Dragendorff's reagent (b); standard of atropine in methanol (left) and standard solution of atropine in methanol tested with Dragendorff's reagent (right) (c)

As it can be seen from Fig. 3b in all of the tested extracts the orange-reddish precipitate was formed, also noticed in an aliquot of atropine standard (Fig. 3c right). This result can be considered as a positive result for presence of alkaloids in the obtained ethanolic extracts. By using LC/MS/MS, in all the obtained ethanolic extracts the presence of atropine is confirmed.

The effects of different parameters on extraction of atropine from *D. Stramonium* seeds are presented in Fig. 4.

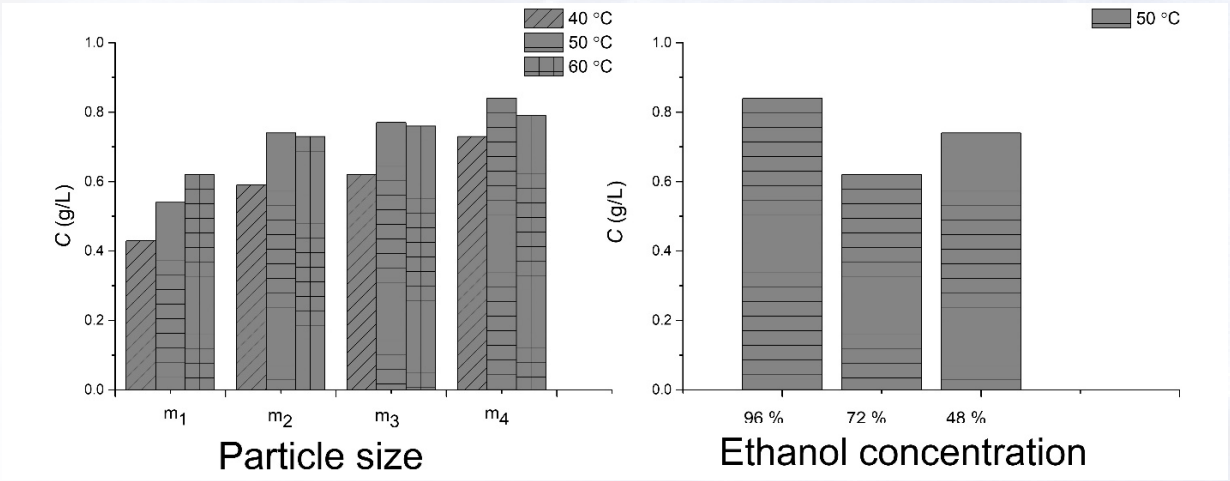


Figure 4. *Effects of different parameters on extraction of atropine from D. Stramonium seeds*

As it can be seen from the results presented in Fig. 4 (left), the concentration of atropine increases with a decrease in particle size (from $m_1$ to $m_4$) at all temperatures (40 °C, 50 °C and 60 °C) with 96.0 % v/v ethanol as a solvent. The highest concentration of atropine is obtained by extraction at the temperature of 50 °C, while the lowest concentration of atropine is observed at the temperature of 40 °C. After determining the most favourable extraction conditions in terms of particle size (dp=1.7 mm ($m_4$)), and the temperature of extraction (50 ° C), further optimization of the extraction process was performed by varying the ethanol concentration. As it can be seen from Fig. 4 (right), in terms of ethanol concentration, using 96.0 % ethanol as a solvent was found to be the most favourable for the atropine extraction.

By perceiving the results of this work and the time required for preparation, the extraction and analysis of atropine in extracts, it is indisputable that the hereby presented conventional heated solid-liquid extraction with tandem mass spectrometry can be a promising tool for forensic analysis of atropine from *D. Stramonium* seeds and various similar plant materials.

## CONCLUSION

The conventional heat-assisted solid-liquid extraction of atropine from *D. Stramonium* seeds was investigated. The effect of temperature, the particle size, and ethanol concentration on the extraction rate of atropine was evaluated, and the optimal conditions were found to be: particle size (dp=1.7 mm), the temperature of extraction (50 °C), and the concentration of ethanol (96.0 % v/v).

The Dragendorff's reagent confirmed the presence of alkaloids in the extracts of *D. stramonium* seeds and proved to be a good preliminary test for atropine. A high concentration of atropine was determined in all of the obtained ethanolic extracts by LC/MS/MS method, which was found to be a sensitive and reliable method for analytical determination of atropine.

Although it is necessary to improve the experimental design and in time to come examine more variable parameters for the optimisation of atropine extraction, the method of the extraction and detection of atropine from *D. Stramonium* seeds presented in this work demonstrated to be fast and efficient. Therefore, this work may be a useful contribution to the future improvement of the methods used for the extraction and analysis of tropane alkaloids in forensic chemistry.

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