OPEN ACCESS
Freely Available Online

JOURNAL OF NEW DEVELOPMENTS IN CHEMISTRY
ISSN NO: 2377-2549

Research Article

DOI: 10.14302/issn.2377-2549.jndc-20-3413

Quantification and Comparison of Opium (Morphine) and Tramadol from Biological Samples "Liquid - Liquid Extraction"

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Abstract

Two analgesic were determined opium (morphine) and tramadol and comparison between two methods of extractions from biological samples.

Opium and its derivatives and tramadol are the most commonly used medications for treatment of acute and chronic pain. opium was used as a sedative and hypnotic, but it was determined to be addictive and tramadol prescribed narcotic analgesic; main metabolite of opium is morphine and tramadol overdose was reported old male 40 years. Morphine and tramadol isolated by two methods of extraction, Stas Otto and ammonium sulfate extraction from liver tissues and comparison between efficiency of the two methods. Liver extractions have morphine and tramadol was quantified by GC-MS. Morphine was determined in liver concentration 176 u/g in Stas Otto. Liver concentration of morphine 267 u/g in ammonium sulfate extraction.

Tramadol was determined in liver concentration 26.18 u/g in Stas Otto. Liver concentration of tramadol 22.41 u/g in ammonium sulfate extraction.

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Citation: Ahmed M.A. Shihata, A.M. ELESSAWY, Alaa M. Elanwer (2020) Quantification and Comparison of Opium (Morphine) and Tramadol from Biological Samples "Liquid - Liquid Extraction". Journal of New Developments in Chemistry - 2(4):29-34. https://doi.org/10.14302/issn.2377-2549.jndc-20-3413

Keywords: Morphine, Tramadol, Extraction,GC-MS.

Received: May 30, 2020 **Accepted:** Jul 15, 2020 **Published:** Jul 25, 2020

Editor: Leli Zeng, College of Pharmacy and Health Sciences, St. John's University, Queens, NY 11439, USA

Introduction

Symptoms of opiod intoxication are similar tramadol analgesics, Symptoms include central nervous system (CNS) depression and coma, tachycardia, cardiovascular collapse, seizures, and respiratory depression up to respiratory arrest.

Opioid is a term for a number of natural substances (originally derived from the opium poppy) and their semi-synthetic and synthetic analogues that bind to specific opioid receptors. The main components of opium is morphine has widespread effects in the central nervous system and on smooth muscle.

Opium was used as a sedative and hypnotic, but it was determined to be addictive, the effects by activating specific G protein-coupled receptors in the brain, spinal cord, and peripheral nervous system. There are three major classes of opioid receptors being delta δ-opioid, kappa κ-opioid and mu μ-opioid. Opium will generate an agonist activity which will later open the potassium channels and prevent the opening of voltage-gated calcium channels. This activity causes a reduction in neuronal excitability and inhibits the release of pain neurotransmitters [1].

Opium and its derivatives and tramadol are the most commonly used medications for the treatment of acute and chronic pain. Opium and its alkaloid-derivatives can also be used as tranquilizers, antitussives and in the treatment of diarrhea [2], but the direct use of opium is not common nowadays and its derivatives such as morphine and codeine etc...are used.

The unspecific effect of opium to the different opioid receptors produce the generation of various effects such as sedation, euphoria, dysphoria, respiratory depression, constipation, pruritus, nausea, and vomiting. It is reported that the secondary effects tend to be diminished as long-term use tolerance is developed. Some reports have also shown an opioid-driven impairment of the hypothalamic function that can result in a loss of libido, impotence, and infertility.

The addictive character of opium is related to the binding to the μ-opioid receptors, which will activate dopaminergic neurons in the ventral tegmental area of the midbrain and thus, enhance the dopamine release in the nucleus accumbens. This mechanism involves the reward activity of the mesolimbic dopaminergic pathway [3].

Opium contains tenths different alkaloid opiates. The most common metabolism of opiates is to be ultimately converted to morphine which is further converted to morphine-3,6-diglucuronide. In the cardiovascular system, there are reports of peripheral vasodilatation, including cutaneous causing flushing of the face, neck, and thorax, impaired sympathetic reflexes and postural hypotension. In the gastrointestinal and urogenital system, the increase in smooth muscle tone has been shown to produce reduced peristalsis, delayed gastric emptying and urinary retention [1].

The main opium overdose toxicity effect is decreased respiratory rate and depth, which can progress to apnea and therefore result in death. Other complications (pulmonary edema, which usually develops within minutes to a few hours after opioid overdose) and death result primarily from hypoxia. Pupils are miotic. Delirium, hypotension, bradycardia, decreased body temperature, and urinary retention may also occur [1].

Tramadol is rapidly absorbed after oral administration. The major metabolites of tramadol are O-monodesmethyltramadol, N,O-didesmethyltramadol and their conjugates, and N-monodesmethyltramadol. O-Monodesmethyltramadol is an active metabolite and has a effective analgesic than tramadol drug. The effective of tramadol due to inhibition of nervous transports such as norepinephrine and serotonin reuptake as well as agonism of mu receptors which cause blocking pain impulses of the spinal cord while the O-desmethyltramadol binding in μ-opioid receptor. This binding wit mu receptors causing effectives as opioid; while noradrenergic pathways causing inhibition of norepinephrine reuptake in Central Nervous System (CNS); Affecting serotonergic pathways causing inhibition of serotonin reuptake in the CNS; GABAergic pathways causing increased GABA neurotransmitter in the brain [4-10].

The relation between serum level and analgesic effect is not the same among different people. It is estimated that the normal therapeutic serum level of tramadol and its metabolite are 0.1 to 0.3 mg/L and

0.03 to 0.04 mg/L, respectively (10-13). It is advised that the drug be used with caution in those with liver or kidney failure, due to metabolism in the liver (to desmetramadol) and elimination via the kidneys [12].

Protein Precipitation is the process in which protein is separated from tissues or any extra contaminants that may be mixed with it. It is an important part of downstream processing and can be done with a variety of different techniques. While there are a number of different methods of precipitation, in this study two methods were used to comparison in extraction opium as morphine and tramadol as free drug.

Materials and Methods

Instrumentation

GC-MS, (Agilent 6890) mass spectrometry detector (Agilent 5973), column HP-5-MS and applying conditions:

- Gas type is helium and pressure 39.8 psi, flow 1.5 ml/min and velocity 32 cm/sec.
- Oven temperature: initial temperature 150 °C and hold time 2 min, rat 25 °C/ min to 280 °C, hold time 20 min and run time 25 min.

Reagents

- Stas Otto extraction: ethyl alcohol, acetone and tartaric acid.
- Ammonium sulfate extraction: saturated ammonium phosphate and HCL acid .
- BSTFA (N, O-Bis(trimethylsilyl) trifluoroacetamide) purchased from Sigma Aldrich.
- Acetonitrile HPLC grade.

Procedure

Weight from biological samples (liver), convert tissues to paste and divide into two equal weights and put in two conical, the first for Stass-Otto and second for Amm. Sulfate. The two samples were leaved to overnight and filtration.

The extract (filtrations) converted to alkaline by ammonia and chloroform solvent was used, addition of each extracted samples BSTFA and acetonitrile, vortex for 60 second then incubate at 70 Celsius for 20 min. Inject 2uL of each specimen into GC-MS.

Result and Discussions

The extraction of drugs from solid tissues requires the tissue matrix be broken down to release drugs into an environment from which they are accessible for solvent extraction. This can be achieved by cutting tissues by scissor and homogenization. Many methods direct solvent extraction are used classic for protein precipitation.

Liquid-liquid extractions are predominates in most laboratories, The choice of an appropriate solvent is often a matter of experience or tradition. The chosen solvent should ideally extract as much of the target analyte as possible.

Many methods of extraction drugs from biological samples were used, but the most popular ones is Salt Induced Precipitation (Salting Out). with ammonium sulfate the first method of "salting out" precipitation protein can occur with a number of different types of neutral salts but ammonium is preferred salt because it is high on the Hofmiester series and has a high solubility rate.

Protein interacts with the salt as opposed to the water, which leads to less interaction between the water and the protein's solvent layer, which in turn leads to more hydrophobic patches being exposed and to encourage those patches to interact with one another. This leads the proteins to aggregate and precipitate. The second methods is Precipitation with miscible solvents, such as ethanol to a solution cause proteins in the solution to precipitate. The solvent layer around the protein will decrease as the organic solvent progressively displaces water from the protein surface and binds it in hydration layers around the organic solvent molecules. With smaller hydration layers, the proteins can aggregate by attractive electrostatic and dipole forces.

Validation Study

Limit Of Detection (LOD)

several calibration were injected in different concentration (500, 200, 100, 50,10 ug) a peak at 100 ug concentration fulfilled the criteria of acceptance for LOD of liquid- liquid extraction concentration as peak noise ratio ≥ 3:1(determined by peak height).

Selectivity: 4 blank samples were prepared and injected and no significant signals were detected in the same retention time of analyte.

Sensitivity

Many calibrations were injected in different concentration (500, 200, 100, 50,10 ug) the response was found to be directly proportional to concentration.

Limitation

samples with concentration less than 10 u/L extraction did not show signal with accepting the method.

Mortality has been reported old male 40 years in Upper Egypt, his family mentioned that he wasn't orally known materials and dead at reached to emergency of hospital. Autopsy didn't find any clear signs and lab investigations of pesticides and insecticides and another routine work of toxic compounds it's negative results and other work of drugs except morphine and tramadol drug by high concentrations was determined (over dose). Opium (morphine) and tramadol and its metabolite O-desmethyltramadol were determined in over dose by two famous methods of extractions and comparison between its Stas-Otto and ammonium sulfate are used to remove fatty components, impurities and other interference materials and precipitate proteins.

The results reported over dose and from figs. 1 and 2 (two figs contain comparison) Stas-Otto had higher extraction efficiency of tramadol and O-desmethyltramadol more than in ammonium sulfate extraction in tramadol and metabolite's,

Liver extractions have morphine and tramadol, Morphine was determined in liver concentration 176 u/g in Stas Otto. Liver concentration of morphine 267 u/g in ammonium sulfate extraction. While tramadol was determined in liver 26.18 u/g in Stas Otto and liver concentration of tramadol 22.41 u/g in ammonium sulfate extraction.

hence Stas-Otto is preferred while neutral salt of ammonium sulfate had higher extraction efficiency of morphine more than in stas otto method extraction in opium and metabolite's, hence ammonium sulfate is preferred

Conclusion

Physicians and experts in branches of medicine must be aware peoples from opium and generally opoides compounds has similar effected such as tramadol addiction and over dose of opium and

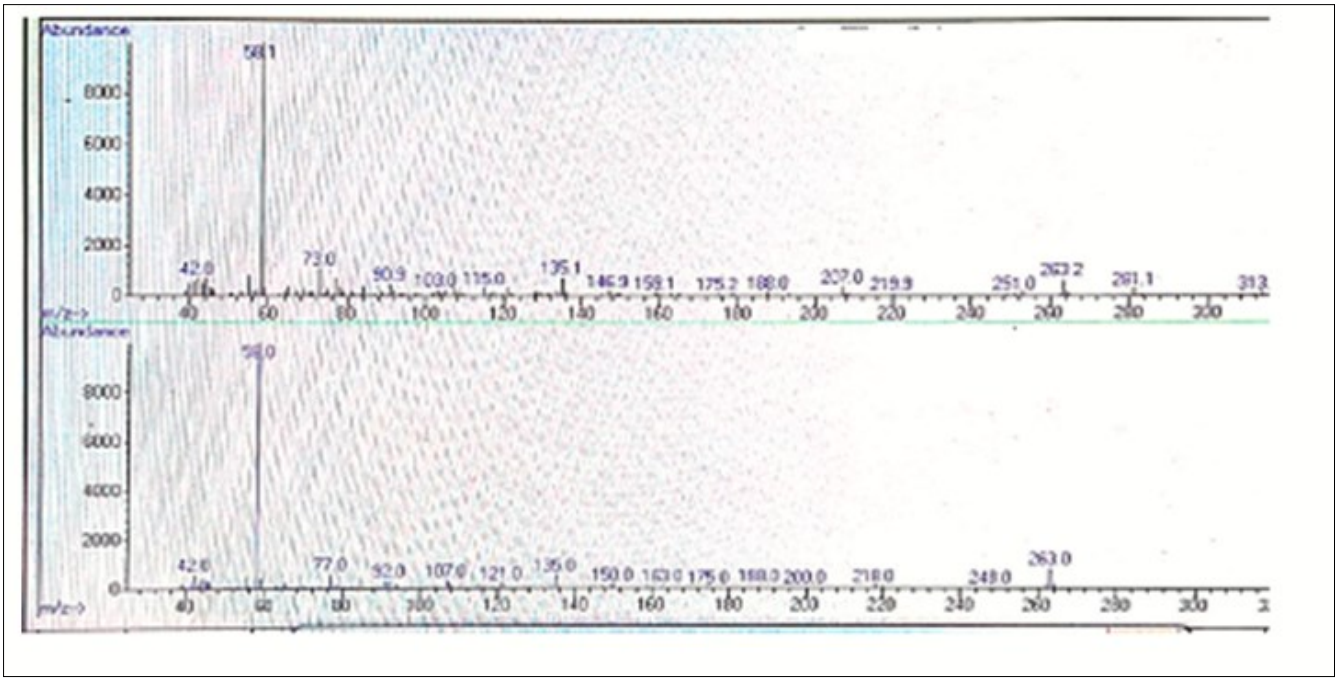

Figure 1. Ion Fragmentation of Morphine m/z (73, 146,196, 236, 429)

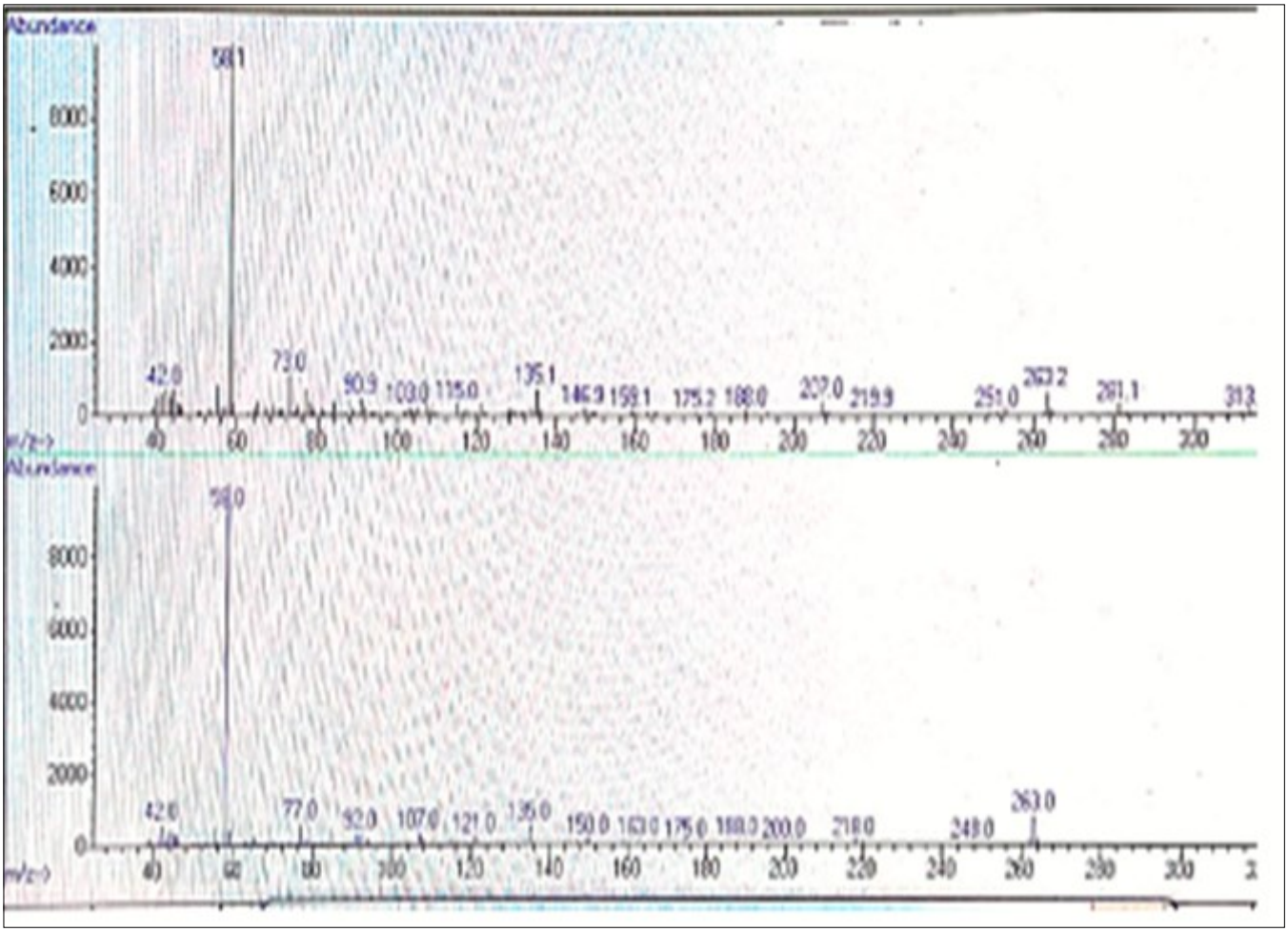

Figure 2. Ion Fragmentation of Tramadol m/z (42, 58, 77, 135, 263)

tramadol, the governments must be but control in used this compound and fights distribution it's materials between peoples specialties youth.

Two methods were used to isolation and precipitation protein (Stass-Otto and Ammonium Sulfate), quantitative of morphine and tramadol respectively are 176 u/g and 267 u/g, and 22.41 u/g and 26.18 u/g in Stas Otto and ammonium sulfate, So that results explained morphine extraction by Amm. Sulfate excellent while tramadol in Stas-Otto is preferred than Amm. Sulfate, and good conditions of GC-MS was used to quantification morphine and tramado.

Objective

Morphine and tramadol quantification in biological samples (liver) by two methods precipitations of protein using GC-MS and discuses methods of prefer by applied in liver tissues of old male 40 years over dose in Upper Egypt.

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