SYNTHESIS & CHARACTERIZATION OF SODIUM OXYBATE AND DEVELOPMENT OF VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR ITS FORMULATIONS

S. Vijayaraj*, R. Gandhimathi, V. Sarathkumar

*Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy,
A. Rangampet, Tirupathi-517102, Andhra Pradesh - 508 206, India.

ABSTRACT

GHB also known as Sodium oxybate has been used in a medical setting as a general anesthetic, to treat conditions such as insomnia, clinical depression, narcolepsy and to improve athletic performance. It is also used as an intoxicant. As drug was not available it was synthesized and used for the present work. The present work was intended to synthesise and characterize the sodium oxybate and develop a method to determine the assay by U.V. Spectrophotometry and validate the method, for both bulk and formulations of Sodium oxybate. This method involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this method has shown accurate and précised results. By these results this method was found to be rapid, simple, accurate, economic method for analysis and quality determination.

KEY WORDS: Sodium oxybate, U.V. Spectrophotometry, γ-Hydroxybutyric acid.

INTRODUCTION

γ-Hydroxybutyric acid (GHB), also known as 4-hydroxybutanoic acid and sodium oxybate is a naturally occurring substance found in the human central nervous system as well as in wine, beef, small citrus fruits, and almost all animals in small amounts. It is also categorized as an illegal drug in many countries. It is currently regulated in Australia and New Zealand, Canada, most of Europe and in the US.

GHB as the sodium salt, known as sodium oxybate, is sold by Jazz Pharmaceuticals under the name Xyrem to treat cataplexy and excessive daytime sleepiness in patients with narcolepsy. GHB has been used in a medical setting as a general anesthetic, to treat conditions such as insomnia, clinical depression, narcolepsy, and alcoholism, and to improve athletic performance. It is also used as an intoxicant (illegally in many jurisdictions) or as a date rape drug. GHB is naturally produced in the human body's cells and is structurally related to the ketone body beta-hydroxybutyrate. As a supplement/drug, it is used most commonly in the form of a salt, for example sodium gamma-hydroxybutyrate (Na.GHB, sodium oxybate, or under the brand name Xyrem.) or potassium gamma-hydroxybutyrate (K.GHB, potassium oxybate). GHB is also produced as a result of fermentation, and so is found in small quantities in some beers and wines.

Succinic semialdehyde dehydrogenase deficiency is a disease that causes GHB to accumulate in the blood. Sodium oxybate (Xyrem) is the sodium salt of gamma hydroxybutyrate (GHB). Xyrem 500mg/ml solutions licensed for the treatment of cataplexy in adult patients with narcolepsy. Cataplexy is an abrupt, reversible decrease in muscle tone caused by emotion, reported by approximately 75% of patients with narcolepsy. The term narcolepsy is used to describe a syndrome comprising of symptoms: cataplexy, hypnologic hallucinations and sleep paralysis Sodium oxybate is indicated for the treatment of cataplexy in adult patients with narcolepsy. It is considered to act in a different way to the only other the licensed medication, clomipramine, for this indication. Cataplexy is an abrupt,

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reversible decrease in muscle tone caused by emotion and is reported in approximately 75% of patients with narcolepsy.

Sodium oxybate is the sodium salt of gamma hydroxybutyrate (GHB) and is a schedule 4 (part 1 CD Benz) controlled drug in the UK. Sodium oxybate is associated with dose-related improvements in the symptoms of narcolepsy and reductions in the number of attacks of cataplexy. The most significant improvements/reductions were seen in patients taking 9g/day. These effects have been demonstrated for treatment periods of 12 months or longer. The most commonly reported adverse effects of sodium oxybate included headache, nausea and dizziness. No withdrawal effects were seen in trials. Abrupt withdrawal leads to a gradual increase in the number of cataplexy attack.

Oxybate (GHB) is a metabolite of γ-aminobutyric acid (GABA) which is synthesised and accumulated by neurons in the brain. It is present at μM concentrations in all brain regions investigated as well as in several peripheral organs, particularly in the gastro-intestinal system. Neuronal depolarization releases GHB into the extracellular space in a Ca2+-dependent manner. A family of GHB receptors in rat brain have been identified and cloned and most probably belong to the G-protein-coupled receptors. High-affinity receptors for GHB are present only in neurones, with a restricted specific distribution in the hippocampus, cortex and dopaminergic structure so frat brain.

In general, stimulation of these receptors with low (physiological) amounts of GHB induce hyperpolarisation in dopaminergic structures with a reduction of dopamine release. However, in the hippocampus and frontal cortex, GHB seems to induced polarization with an accumulation of Cgmp and an increase in inositol phosphate turnover. However, at higher (therapeutic) exposures, GHB receptors are saturated and probably desensitized and down regulated. Such GHB dopaminergic hyperactivity, strong sedation with anaesthesia and EEG changes those are consistent with normal sleep. The pathogenesis of narcolepsy is still unknown, but an imbalance between monoamines and acetylcholine is generally accepted.

Recent research has found a marked reduction of the neuropeptide hypocretin type 1 in the cerebrospinal fluid of a majority of patients and a global loss of hypocretins in postmortem brain tissue of narcoleptic subjects. The hypocretins are synthesized by a small group of neurons predominantly located in the lateral hypothalamic and perifornical regions of the hypothalamus. The hypothalamic system directly and strongly innervates and potently excites noradrenergic, dopaminergic, serotoninergic, histaminergic and cholinergic neurones.

The effect of GHB on this system has not been investigated. However, the available data indicate that its mode of action is likely to relate to non-specific dopaminergic stimulation rather than the hypocretin system. Formal nonclinical pharmacology studies to investigate the primary pharmacodynamics have not been conducted by the applicant, rather a comprehensive review of the scientific literature has been conducted. The publications included have been selected based on their relevance to the proposed indications, based on evidence of efficacy from early clinical studies.

In addition, animal models of cataplexy and narcolepsy are continuing to be developed, but have not yet been fully validated. Little nonclinical information is available on its effects on narcolepsy in general, and cataplexy in particular. Available, directly relevant data, from the published literature, has been reviewed but the current understanding of the role of GHB in the CNS does not provide a mechanistic explanation of the positive clinical effects reported in the dossier.

GHB had no effect on cataplexy in dogs with hereditary narcolepsy when administered as a single dose of 500mg/kg i.v. or 50mg/kg/day p.o. for 3 consecutive days. However, although such dogs have amutation of the type 2 hypocretin receptor, the clinical relevance of this model remains to be established. Moreover, a dose of 75mg/kg/day for atleast 14 days is required for efficacy in humans. Though the precise mode of action is unknown, these sedative properties of GHB and its effects on sleep may play a role in the efficacy observed in humans. Evidence from a human clinical study (Study OMC-SXB-20) where GHB was administered to narcoleptic patients and overnight poly somno grams(PSG) were recorded, suggests that GHB modifies sleep architecture, specifically a dose-related increase in Stage 3 & 4 slow wave sleep (SWS,deltasleep). The cause of human narcolepsy and cataplexy is, as yet, unknown. Recent evidence points to the loss of hypocretin-containing neurones, possibly due to autoimmune attack, as a likely cause (Scammell 2003). Hypocretin is a neuro transmitter that has roles amongst others, in sleep-wake regulation. Alterations in hypocretin neurotransmission have also been observed in mouse and models of narcolepsy, although no studies have been undertaken with GHB in these models.

GHB reaches much higher concentrations in the brain and activates GABAB receptors, which are primarily responsible for its sedative effects. GHB receptors are densely expressed in many areas of the brain, including the cortex and hippocampus, and these are the receptors that GHB displays the highest affinity for. There has been somewhat limited research into the GHB receptor; however, there is evidence that activation of the GHB receptor in some brain areas results in the release of glutamate, the principal excitatory neurotransmitter. Activation of both the GHB receptor and GABA (B) is responsible for the addictive profile of GHB. GHB's effect on dopamine release is biphasic, low concentrations stimulate dopamine release via the GHB receptor Higher concentrations inhibit dopamine release via GABA(B) receptors as do other GABA(B) agonists such as baclofen. After an initial phase of inhibition, dopamine release is then increased via the GHB receptor. This explains the paradoxical mix of

sedative and stimulatory properties of GHB, as well as the so-called "rebound" effect, experienced by individuals using GHB as a sleeping agent, where in they awake suddenly after several hours of GHB-induced deep sleep. That is to say that, overtime, the concentration of GHB in the system decreases below the threshold for significant GABAB receptor activation and activates predominantly the GHB receptor, leading to wakefulness [1-5].

GAMMA BUTYRO LACTONE
Fig 1. Structure of Gamma Butyro Lactone
:
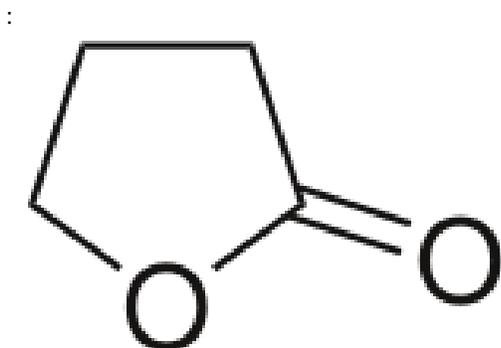
Moleculer formula   : $C_4H_6O_2$
Moleculer weight   :   86.09
IUPAC Name   :   Dihydrofuran-2(3H)-One
Description : One of the FURANS with a carbonyl thereby forming a cyclic Lactone. It is an endogenous compound made from gamma-aminobutyrate and is the precursor of gamma-hydroxybutyrate. It is also used as a pharmacological agent and solvent.
Solubility: Freely soluble in water & organic solvents
P^H : 12
Category : Adjuvants, Anesthesia
Dose : 1.5gms

MATERIALS AND INSTRUMENTS
Precursor
    Gamma butyro lactone was purchased from sigma Aldrich Bangalore.
Chemicals and solvents
- Methanol (Analytical Grade)
- Sodium hydroxide
- Ethanol

Glassware
    Beakers, measuring cylinder, pipettes, petridish plates, hot air oven, Buchner funnel etc., were used for synthesis.

Instruments used
- SHIMADZU UV Pharmspec Spectrophotometer 1700 UV-Visible Spectrophotometer connected to a computer loaded with spectra manager software UV Probe (Spectral band width of 1nm and

wavelength accuracy of ±0.3nm with a pair of 10mm matched Quartz cells)
- SHIMADZU (ELB 300) Electronic balance
- SHIMADZU (BL 22OH)Electronic balance
- TOSHIBHA (India) Ultra sonicator

EXPERIMENTAL SECTION
Synthesis of Sodium Oxybate
    Weigh accurately about (5gm,0.25moles) of NaOH was dissolved in 83ml of ethanol and once cooled GBL (10ml,0.26moles)was added in 2.5mlfractions.a white precipitate of NaGHB immediately formed and the solution was allowed to stand for 2hrs before the precipitate was collected by filtration and recrystalization through a Buchner funnel. The precipitate was then transferred to a glass dish and dried for 3hrs in elective oven at 40^0c.The dried weigh powder (13.2g.,50%) was immediately transferred to a glass air tight screw top storage container [6-9].
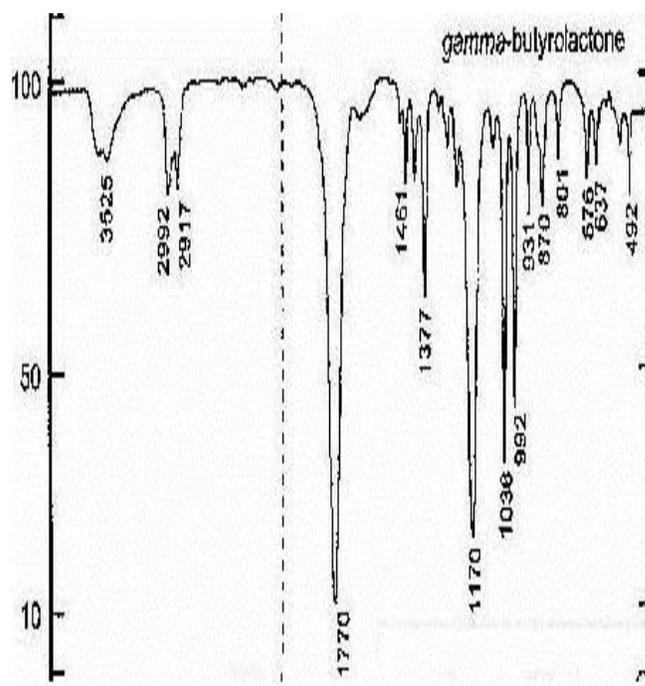
Fig 2. Synthesis of Sodium Oxybate
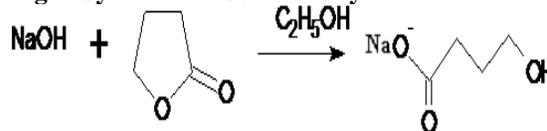
Sodium   Gamma butyro   Sodium oxybate
hydroxide   Lactone

Characterization of Sodium Oxybate
Fig 3. FT –IR Spectra of Gamma- Butyrolactone

Table 1. Interpretation data of Gamma Butyro Lactone

Vibrational Mode	Wavenumber (cm^{-1})	Strength of Peak
Carbonyl stretching mode	1770	Very strong
OH bond stretching	3525	Relatively weak
C-H bond	2992	Relatively weak
In plane deformation of the carboxyl hydrogen and stretching of the carbon oxygen bond in the carboxyl group	1450-1150	Strong
Stretching C-O bond of the terminal hydroxyl group	1038	Strong

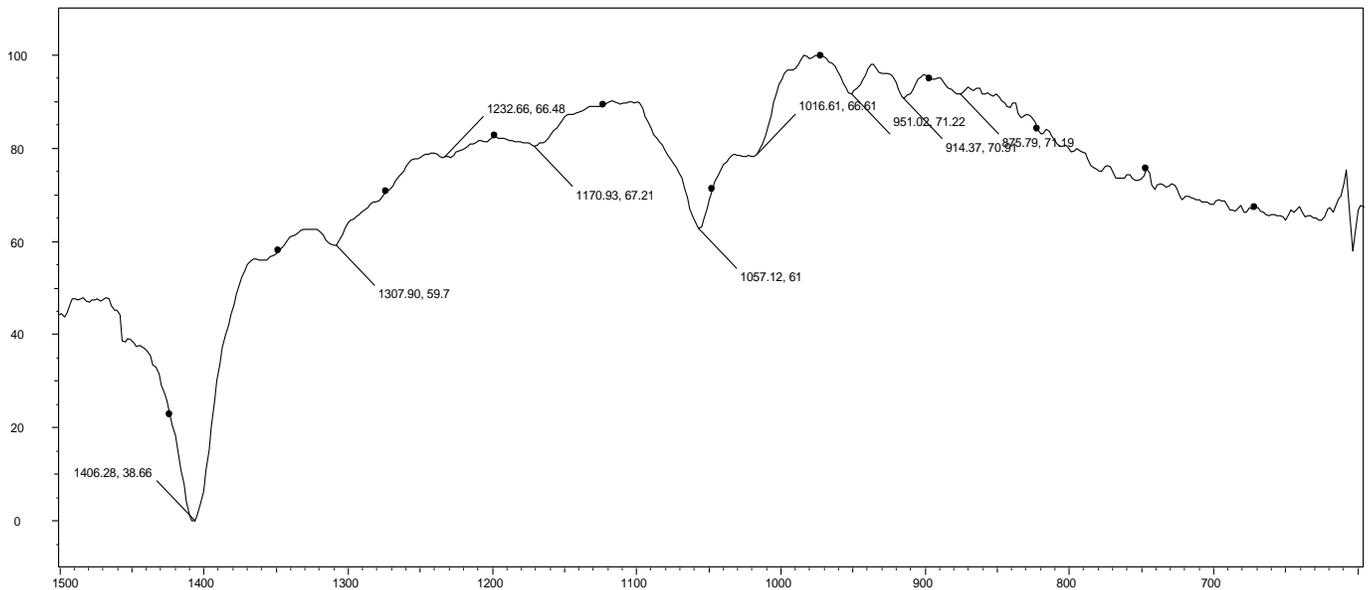**Fig 4. Sodium Oxybate: (FTIR)**
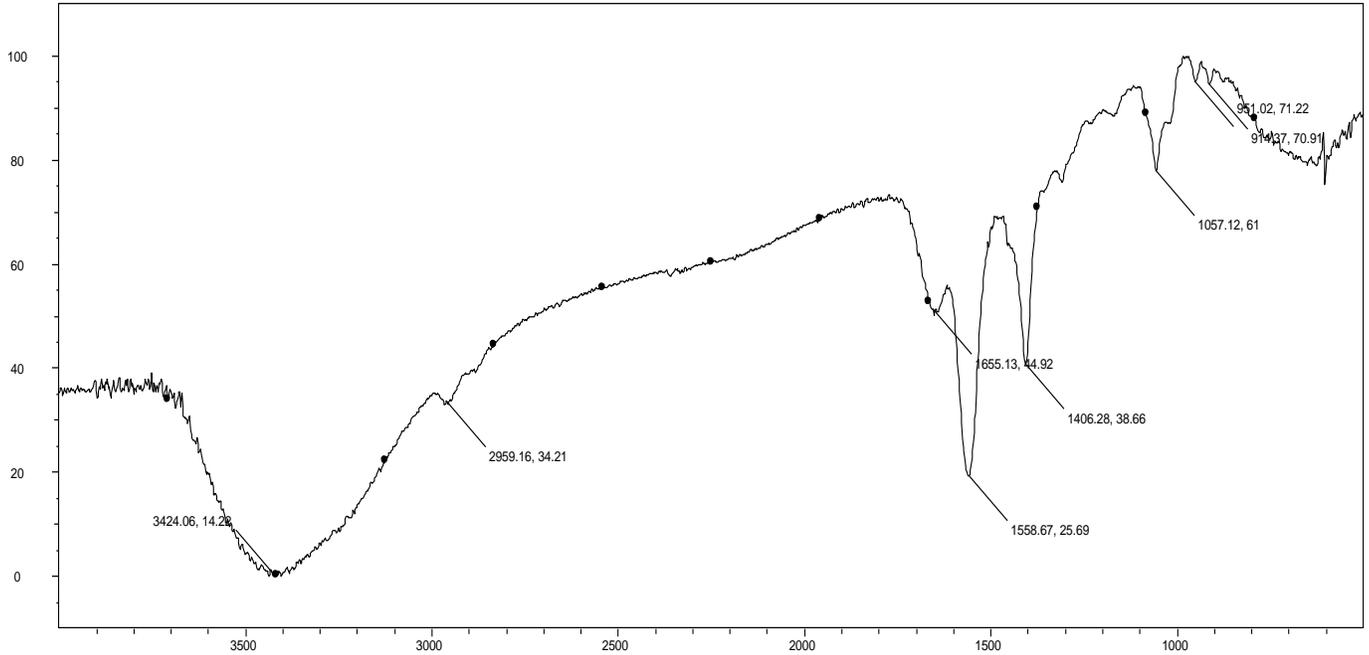
Fig 5. Finger Print Region

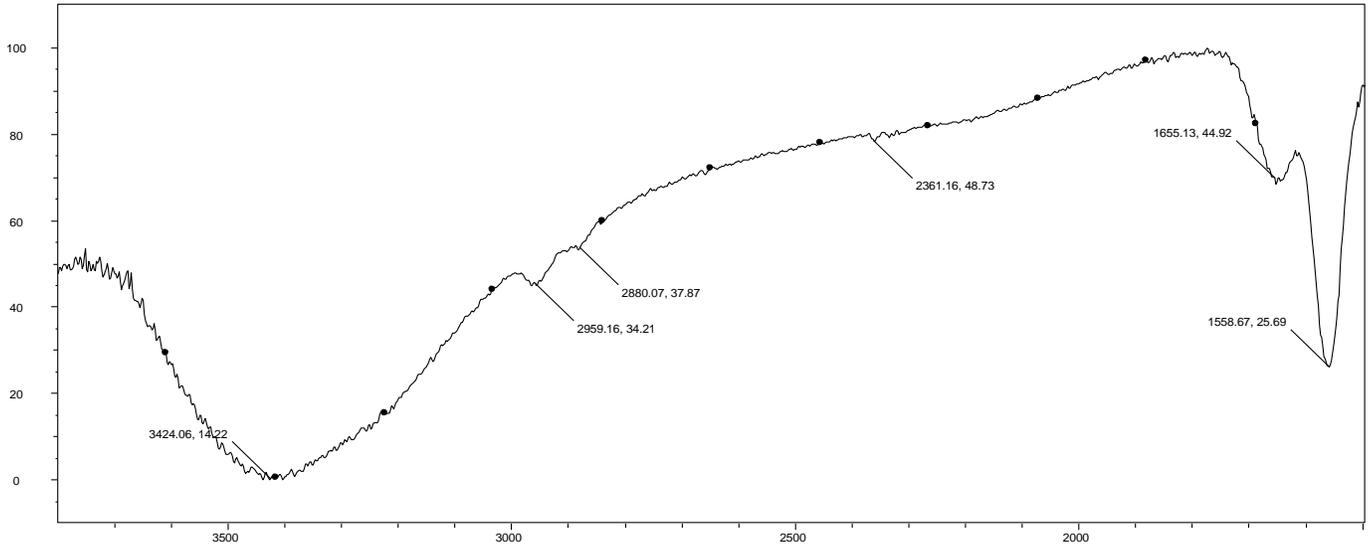**Fig 6. Functional group region**

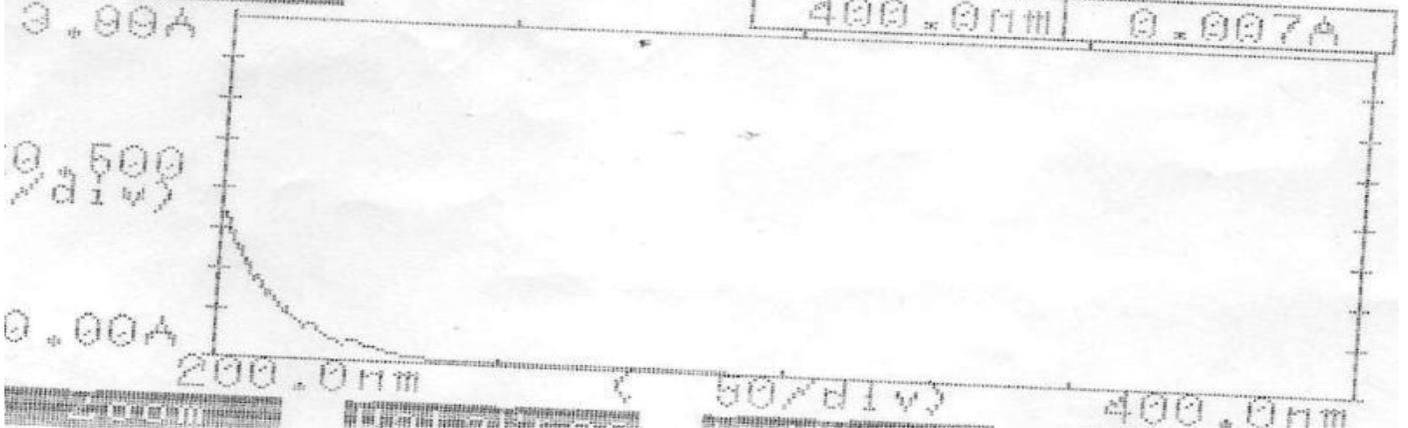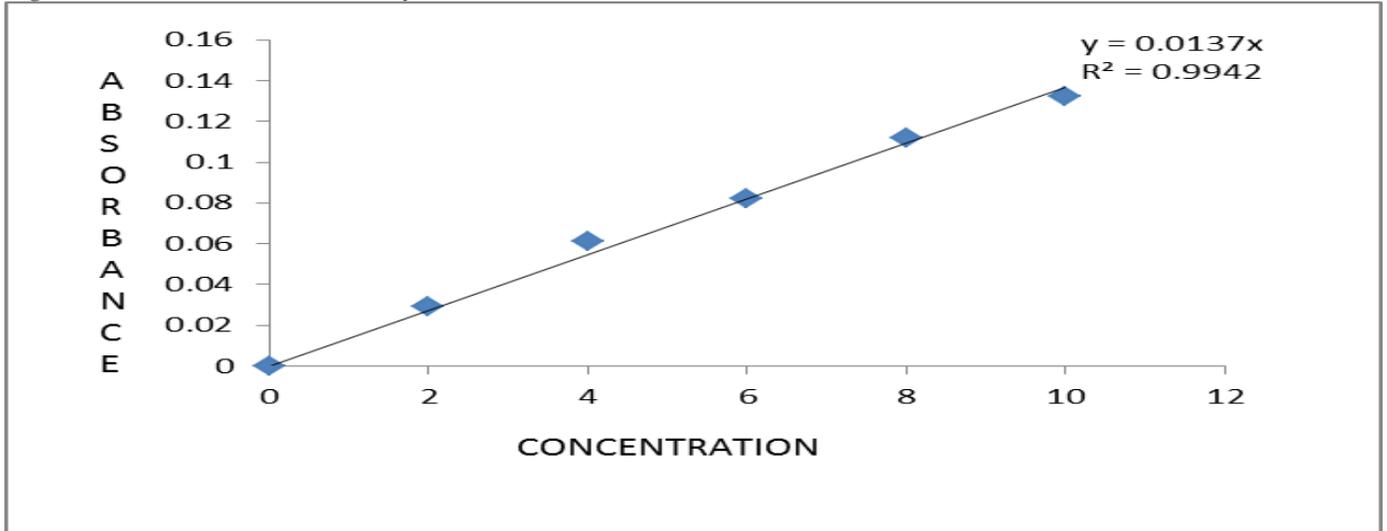**Fig 7. UV Spectrum of Sodium Oxybate in Methanol**

Fig 8. Standard curve of sodium oxybate

Table 2. Interpretation Data of Sodium Oxybate

Vibrational Mode	Wave number (cm^{-1})	Strength of Peak
OH bond stretching	3318	Relatively weak
C-H bond	2959	Relatively weak
Carbonyl stretching mode	1555	Very strong
In plane deformation of the carboxyl hydrogen and stretching of the carbon oxygen bond in the carboxyl group	1156	Strong

Table 3. standard curve values

Concentration(µg/mL)	Absorbance
0	0
2	0.029
4	0.061
6	0.082
8	0.112
10	0.132

Table 4. Method Validation Parameters of sodium oxybate

Parameters	Results
λ_{max}	205 nm
Beer's law limit	2-10 µg/ml
Molar absorptivity	3.1456 x 10^{-4} L mole^{-1} cm^{-1}
Regression equation (Y = mx + c)	y = 0.013x + 0.00333
Slope (m)	0.013
Intercept (c)	0.00333
Correlation coefficient (r)	0.994
Relative standard deviation (%)	0.099
LOD Value	0.11609µg/ml
LOQ Value	0.42522 µg/ml

METHOD DEVELOPMENT AND VALIDATION OF UV METHOD
Selection of solvent
Solubility of drugs was checked in several solvents like ethanol, methanol and some buffers and then UV-spectra of drugs in these solutions were recorded. Absorbance of the drug was higher and both drugs exhibited distinct λ_{max} in methanol and hence methanol was selected as solvent for further studies [10-13].

Selection of wavelength
A wavelength which gives good response for the drug to be detected is to be selected by trial and error method. From the UV spectra 205 nm was selected as the wavelength for study, fig.6.

Calibration curve preparation
Calibration curve or standard curve is a very important parameter for method validation and assay procedure for the substance. And this factor can be useful to estimate the assay value and drug content present in particular formulation of the drug. This calibration curve linearity can help to detect weather the proposed method is perfect or not.

Calibration curve data were constructed in the range of the expected concentrations of 2µg/ml to 10µg/ml. *Beer's* law was obeyed over this concentration range. The regression equation was determined by using

y =mx+c

Here 'C' is concentration of analyte.

By this calibration curve we can analyse the linearity of the method and range of the method.

PROCEDURE
Preparation of the stock solution
An accurately weighed 10mg quantity of sodiuim oxybate was transferred into a 10 ml volumetric flask. To this, 4ml of methanol was added and the flask was shaken for 1 mins to solubilise the drug and the volume was made up to the mark with methanol to get a standard stock solution of 1mg/ml. This stock solution used for further dilutions and by using distilled water as solvent for estimation.

Preparation of the test solution
Take 100 mg of the sodium oxybate drug (sample) and transferred into a 100 mL volumetric flask. To this, 40ml of methanol was added and the flask was shaken for 5

mins to solubilise the drug and the volume was made up to the mark with methanol to get a standard stock solution. By using this solution the concentrations of 2, 4, 6, 8 and 10 µg/ml solutions were prepared for further validation parameters.

VALIDATION OF THE METHOD

The developed method was validated in terms of parameters like precision, linearity and LOD, LOQ etc [14-18].

Linearity and Range

Sodium oxybate was found to be linear in the concentration range of 2- 10 µg/ml. The absorbances of these solutions were noted at the selected wavelength, 205nm. Calibration curve was plotted using concentration Vs absorbance. At a wavelength of 205 nm- slope, intercept and correlation coefficient values were found to be 0.013, 0.00333 and 0.994, respectively.

Precision

Precision of method was demonstrated by
a)   Intraday precision
b)   Interday precision

Intraday precision

Intraday precision was done by carrying out analysis of standard drug solution at one concentration in the linearity range for three times on the same day and %RSD was calculated.

Inter day precision

Inter day precision was done by carrying out the analysis of standard drug solutions at one concentrations in the linearity range for three days over a period of one week and %RSD was calculated.

Accuracy

Accuracy is the percentage of analyte recovered by assay from known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD (limit of detection)

LOD is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve using following equation:

$$LOD = 3.3 \ \sigma/ S$$

Where σ is standard deviation of Y-intercept and S is slope of calibration curve. The LOD was found to be 0.11609 µg/ml.

LOQ (Limit of quantification)

LOQ is defined as the lowest concentration of

calibration curve that can be measured with an acceptable accuracy, precision and variability. The value of LOQ was determined using following equation:

$$LOQ = 10.3 \ \sigma/ S$$

LOQ (Limit Of Quantification) Value is the minimum quantity of drug that can be quantified by the instrument and the value was found to be 0.4252 µg/ml.

Beers Limit

The limits in which beers law obeyed is beers limit. In the UV method development the accuracy, precision the ruggedness, robustness is showed within range called Beers limit. And for Sodium oxybate, the beers limit range found to be 2µg/mL to 10µg/ml. Within this range the drug shows accuracy, linearity, precision, ruggedness, robustness

Molar Absorptivity

This is the important factor for determining the absorptive property of a drug in 1 mole concentration. And this value can be useful in determining the absorbance of drug in molar concentrations. This for identifying the shifts of the maximum absorbance of the drug during the method development. The molar absorptivity of the Sodium oxybate was found to be $3.1456 \times 10^4 \ L \ mole^{-1} \ cm^{-1}$.

RESULTS AND DISCUSSION

The sodium oxybate is synthesized and characterized by taking FT-IR spectra of gamma butyro lactone and our drug. Gamma butyro lactone can be used as a precursor for this synthesis. By FT-IR studies we can observe the vibrational changes and thus we can characterize the sodium oxybate.The yield of sodium oxybate obtained from preparation using gamma butyro lactone was found to be 13.2gms (50%).

For the UV, Spectroscopic conditions were optimized to get best correlation coefficient and LOD, LOQ. The optimum wavelength for detection and quantification was 205 nm. There was no interference from the diluents, excipients present in the pharmaceutical formulation. To check the Linearity, standard calibration curve of the drug was constructed by plotting using absorbance vs. concentration of standard solution and the curve showed good linearity over a concentration range of 2-10µg/ ml. The regression equation of the drug was found by plotting absorbance (y) vs. Concentration(x) µg/ml. The LOD and LOQ values of sodium oxybate was found to be 0.11609 µg/ml   and 0.4252 µg/ml respectively. The Precision of the method was determined by repeatability (intraday) and intermediate precision (inter day). Precision was expressed as the RSD of the results. The value obtained for the precision studies indicates good repeatability and low inter day variability.

CONCLUSION

The sodium oxybate is synthesized and characterized by taking FT-IR spectra of gamma butyro

lactone and our drug. Gamma butyro lactone can be used as a precursor for this synthesis. By FT-IR studies we can observe the vibrational changes and thus we can characterize the sodium oxybate. The yield of sodium oxybate obtained from preparation using gamma butyro lactone was found to be 13.2gms (50%). The results show that within the concentration range tested, there was excellent correlation between absorbance and concentration. Sodium oxybate was found to be linear in the range of 2 to 10 μg/ml. The LOD of Sodium oxybate was found to be 0.11609 μg/ml. The LOQ of Sodium oxybate was found to be 0.4252 μg/ml. Precision of the developed method was studied under intraday precision; inter day precision. Low % RSD values show that the developed method is precise.

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