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# CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC ANALYSIS OF PANCHRONIUM BROMIDE

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**ABSTRACT:** The present investigation is centered on drug pancuronium bromide, which is a muscle relaxant basically used in clinical practice for an aesthetic purposes. Few rape cases have been reported where in this drug was used to incapacitate the rape victim. All this highlights the abuse potential of *Pancuronium bromide* in crime related to rape. Testing/analyzing *Pancuronium bromide* by making use of body material and other traces present in the surrounding of victim/crime scene will be of great importance for medico legal purpose and forensic view point. In the present study an attempt has been made to analysis *Pancuronium bromide* by chromatographic (TLC and HPLC) and UV-SPECTROPHOTOMETRY. In spite of the limitation the conclusions reached in the present study are of considerable forensic significance.

**Keywords:** Pancuronium Bromide, TLC, HPLC

## INTRODUCTION

Pancuronium bromide is a non depolarizing, neuromuscular blocking agent. It is a chemical compound used in a medicine with brand name PAVULON ( organ international ) , NEOCURON 4mg/2ml , PANURON 2mg/ml . Neocuron is the derivative of the alkaloid ( malouetin ) from the plant MALOUETIA BEQUAERTIANA. *PANCURONIUM BROMIDE* is a typical non depolarizing curare mimetic muscle relaxant (a paralytic agent ). In small clinical dose of *Pancuronium bromide* at low frequency of stimulation, non-depolarizing muscle relaxants act predominantly at the nicotinic receptor site to complete with acetylcholine. In larger doses, some of these drugs also enter the pore of the ion channel to cause blockade. This further weakens neuromuscular transmission and diminishes the ability of acetylcholinesterase inhibitors to antagonize non depolarizing muscle relaxants. Non depolarizing relaxants may also block the prejunctional sodium – but probably not calcium-channels. As result, these muscle relaxants also interfere with mobilizing of acetylcholine at the nerve ending. That blocks the action of acetylcholine at the motor end plate of the neuromuscular junction . Binding of acetylcholine to receptor on the end plate causes depolarizing and contraction of the muscle fibres, non depolarizing neuromuscular blocking agents like pancuronium stop this binding from taking place.

Chemical name of *Pancuronium bromide* is 1-1 (3ά, 17β-diacetoxy-5ά-androstan-2β, 16β-ylene) BIS [1-methylpiperidinium] di-bromide and chemical structure in (Fig-1 and,2).

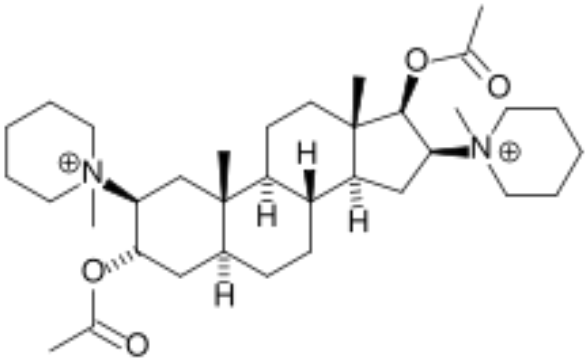

**Fig-1: Chemical structure of Pancuronium Bromide**

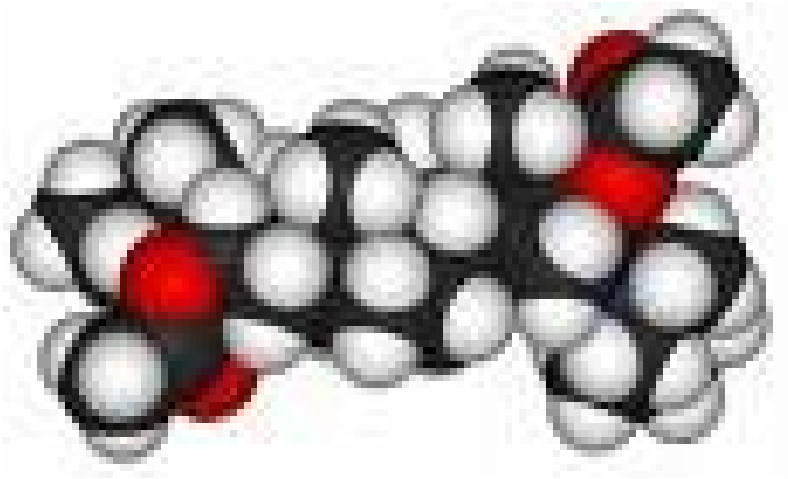

**Fig-2: 3-D view of computer generated structure of Pancuronium bromide.**

Various analytical methods have been developed by several laboratories to analyse *Pancuronium bromide* in aged autopsy tissue and fluids. Several reports are available where qualitative, semi quantitative or quantitative analysis of the autopsy tissues for *Pancuronium bromide* has led to successful investigation of criminal cases. Low cost methods includes colour, (or spot) test, TLC, spray visualization methods, and micro crystal tests. Traditional instrumental method such as Gas chromatography (G.C) G.C/M.S, (I.R) spectroscopy and ultraviolet (U.V), comprise moderate cost instrument techniques. That often requires extraction and with G.C derivitization of both low cost and high cost methods of analysis is addressed [1, 2].

Clarke suggested a chemical test for the presence of *Pancuronium bromide* by dry some antimony chloride over phosphorous penta chloride, melt the dried material at 73C (melting point) and pass dry chloride gas into melted part until the yellow fuming liquid is obtained. Add this liquid to ten times in suspected sample if green colour appears, the presence of *Pancuronium bromide* is indicated [3]. In 1990 the drug with potassium iodide, extracting the complex and submitting the extract to the TLC (Thin layer chromatography) clean up then to GC-MS analysis in the selected ion monitoring mode was conducted.

The two quaternary amines of Pancuronium appears to undergo pyrolytic N – demethylation in the injection port to yield an entity amenable to capillary column gas with additional fragments of 292,323,338,396 and 397 m/z ,each of which was monitored . Isolation and identification and identification in aged autopsy tissues and fluids were made by [4, 5]. In 2005 a novel protocol was optimized for the isolation of the target drug in highly decomposed tissues. Solid phase extraction (SPE) cartridges containing styrene-divinylbenzene were investigated. The semi-purified SPE samples were pre-screened by pyrolysis GC-MS. a candidate specimen was then confirmed by micro bore high performance liquid chromatography /electro spray-ionization/ mass spectrometry with a triple-quadrupole mass spectrometer. The developed procedures provided a qualitative or semi quantitative basis for the investigation of difficult cases involving overdoses of polar drugs [6].

## MATERIAL AND METHODS

The injectable form of Pancuronium bromide was purchased from the medical store. Table no.1 gives the description of the injection. And this injectable form of *Pancuronium bromide* is directly spotted on the TLC (Thin layer chromatographic) plate with the help of capillaries. The TLC chamber to be used in the experiment was washed thoroughly with running tap water, rinsed with distilled water and then dried. Sample was spotted on the TLC plate with the help of fine capillaries. The spots were allowed to dry before being run in the solvent system [7,8]. The spotted plate was developed with the solvent system {Ammonium formate (1): Formic Acid (5): Water (95): Tetrahydrofuran (233)} [3].

After a run of 10 cm the plate was removed from the chamber and allowed to dry in air. TLC plates were observed in day light, UV light and iodine fumes. The hRf value of spots was noted. The developed and marked spots from the plates were scraped with the help of the sharp object and it was transferred on a filter paper. The brown colour due to iodine was allowed to disappear and white silica gel containing the drug was transferred inside a clean test tube. Distilled water (=5ml) shaken well for 5min and then centrifuged. The supernatant portion was used in the present investigation for development of a HPLC-UV method.

Analysis of *Pancuronium bromide* sample was performed on WATER(R) HPLC system comprising 515 binary pumps, 2487 dual wavelength absorbance detector and manual Rheodyne injector. Ac-18 column {spherisorb(R), 250x4.6mm, 5micron} c-18 guard column was placed before the analytical column. The data was acquired and processed in millennium software version 2.1. The mobile phase was filtered through Nylon membrane (0.45micron) using Millipore filter assembly and degassed using ultra Sonicator bath (ELMA 507/H) [9]. 20microliter sample duly eluted and extracted from TLC was injected into the port. The UV absorption spectrum of the drug sample was recorded on BECKMAN(R) DU-400B series spectrophotometer [10].

For HPLC, the detection wavelength was set at 210nm. The various mobile phases flowing at rate of 0.5ml/min employed for the detection of the drug are summarized in {Table no.2}. The mobile phase used at the beginning of analysis i.e. composition no 1 eluted the drug at 4.27min. In order to delay the retention time of drug to avoid interference with the solvent peaks, water was decreased (composition no.2) but no change in the retention time was observed. Further decrease in water content also did not affect the elution time. Change in pH of the drug solution to alkaline range elimination of glacial acetic acid from the mobile phase (composition no.4) or elimination of acetonitrile and glacial acetic acid (composition no.5) however distorted the peak shapes. This suggested that glacial acetic acid is required for the better peak shape of the drug. Finally a simple 1%v/v solution of glacial acetic acid in water (Composition no.6) eluted the drug at 8.9min with no interference with the solvent peaks. The optimized chromatographic conditions for determination of *Pancuronium bromide* were determined Table no.3.

**Table: 1: Chemical information**

| S.NO | Generic name, Code name | Chemical Name | Form | Amount | Manufacture |
|---|---|---|---|---|---|
| 1. | Neocuron | Pancuronium Bromide | Injection | 4mg/2ml | Neon lab LTD Boisar Road, Thane (M.S). |

**Table no.2: Mobile Phase Composition**

| Composition no. | Acetonitrile(ml) | Methanol(ml) | Water(ml) | Glacial Acetic Acid(ml) |
|---|---|---|---|---|
| 1. | 20. | 5. | 74. | 1. |
| 2. | 20. | 10. | 69. | 1. |
| 3. | 20. | 15. | 64. | 1. |
| 4. | 20. | 10. | 70. | - |
| 5. | - | 30 | 70 | - |
| 6. | - | - | 100 | 1 |

**Table no. 3 : Optimised Chromatograhic Conditions**

| | |
|---|---|
| Mobile Phase | Glacial acetic Acid : water (1:100) |
| Flow Rate | 0.5ml/min |
| Column | C18 (spherisorb(R), 250mmx4.6mn,5micrometer) |
| Injection Volume | 20microliter |
| Detection wavelength | 210nm |

## RESULT AND DISCUSSIONS

*Pancuronium bromide*, a smooth muscle relaxant is highly saturated molecules with only isolated double bonds. It absorbs very poorly in the UV region at wavelength of 210nm and hence, numerous methods are available for determination of this drug in formulations and biological fluids these include LC-MS [11, 12], electro analytical detection [13], detection ELSD [14] only one HPLC method using UV detection is reported where the sensitivity is 0.4mg/ml of the drug[15]. During the course of this investigation the commercially available preparation was eluted through TLC by using {Ammonium formate (1): Formic Acid (5): Water (95): Tetrahydrofuran (233)} {3} as a solvent system. The plate was dried and exposed to iodine fuming for the purpose of visualization of eluted spot. This non-destructive method of visualization was found to be most suitable form the aspect of subsequent instrumental analysis for determination of drug in injection. Hence the present study was undertaken to develop a new HPLC method with UV detection and with improved sensitivity. The mobile phase reported by Zecevic et al. [15] was taken as reference and replacing TFA to glacial acetic acid modified it.

The elution time of drug was found independent of acetonitrile or methanol concentration. Thee pH of the drug solution was varied to render the drug completely un-ionized to completely ionize. Also did not affect the elution time. In present investigation glacial acetic acid was found to greatly affect the peak shape and elution time of the drug. The optimum resolution and peak shape was achieved with mobile phase composed of 1%v/v solution of glacial acetic acid at flow rate of 0.5ml/microliter. Though no calibration plot was prepared but analysis of serial dilution of the commercially available injection (neucron) revealed the sensitivity of the method to be 2mg/ml of the drug.

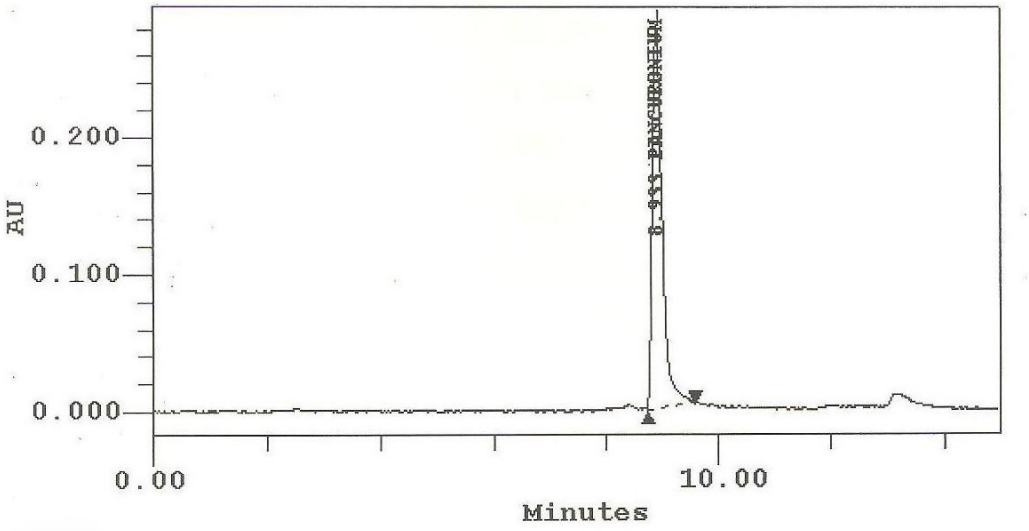


**Fig 3: Chromatogram of Pancuronium Bromide**

## CONCLUSION

The present study has been undertaken to develop a new HPLC method with improved sensitivity. The commercially available formulation of *Pancuronium bromide* was eluted and extracted with thin layer chromatography. The modified HPLC mobile phase comprising of glacial acetic acid: water (1:100) was found to be stable for the elution of pancuronium bromide. Analyses of serial dilution of commercially available preparation revealed the sensitivity of the method to be 2mg/ml.

## REFERENCES

1. Frederick P. Smith A. Jay Siegel {2005}: Hand book of forensic drug analysis, Elsevier Academic Press Publication U.S.A.
2. L.S Goodman, A. Gilman {2006}: The pharmaceutical basis of therapeutics. Mac millan. New York.
3. Clarke EGC {1986}. Isolation and identification of drugs, vol.2 CRC London, UK: The pharmaceutical press, PP 221.
4. Agostan selal {1992}: Clinical pharmokinetics of neuromuscular blocking drugs, clinical pharmokinetics CRC Press. 22:94.

5. Edward J Briglia, Paulette L. M. S.; Michael Katz, M.S.; and Leo A. Dal Cortivo {1990} Attempted murder with Pancuronium Bromide. Journal of Forensic Science, vol. 35. NO. 6, pp. 1468-1476.
6. Brain D. Armando Alcaraz, M.S; and Patrick M.Grant, {2005}. The application of *Pancuronium bromide* (Pavulon) forensic analysis to tissue samples from an angle of death investigation. Journal of Forensic Science, Vol. 50, no.1.
7. Brain D. Armando Alcaraz, M.S; and Patrick M.Grant, {2005}. *Pancuronium bromide* (Pavulon) isolation and identification in aged autopsy tissue and fluid, Journal of Forensic Science, Vol. 50, no.1.
8. Sthal. Egon {1969}. Thin layer chromatography, A laboratory hand book; second edition, springer-verlegn Berline Heidebereg New York. Pp 41-55.
9. M. Weindlmayr-Goettel, E.P. L ankmayr, G. Koberl and H. Gilly {2004}. Determination of vancuronium and pancuronium and its metabolites in human and animal plasma using HPLC and post-column ion-pair extraction with fluorimetric detection. Journal of Analytical Chemistry vol.343, no. 1.
10. Salem, A. {1997}: Analysis of buprenorphine in rat plasma using a solid –phase Extraction technique and HPLC with electrochemical detection, J. pharmacology and toxicology methods, 37:75-81.
11. Kerskes et al. {C.H.M. Kerskes, K.J. Lusthof, P.G.M. Zweipfenning, J.P. Franke, {2002}. The detection and identification of quaternary nitrogen muscle relaxants in biological fluids and tissues by ion-trap LC-ESI-MS, J. Anal. Toxicol. 26-29-34.
12. Kiyotaka Usui, Takanori Hishinuma, Hiroaki Yamaguchi, Toshihide Saga, Toshihiro Wagatsuma, Kunihiko Hoshi, Naoki Tachiiri, Kuniaki Miura and Junichi Goto. {2006}. Simultaneous determination of pancuronium, vancuronium and their related compounds using LC-ESI-MS. Journal of Analytical Chemistry vol 8. pp. 166-171.
13. Alaa A. Salem, Barsoum N. Barsoum, Dalia M.El Said {2005}. Rapid determination of gallamine triethiodide {flaxedil} and *Pancuronium bromide* {Pavulon} in pharmaceutical and urine matrices by mean of modified-carbon-paste ion-selective electrodes. 861-872.
14. Analysis of muscle relaxants by HPLC using ELSD. www.discoveryscience.com/productinfo/technical/app/0046e. Date: 09.06.07.
15. Zecevic M.; Zivanovic L.J.; Stojkovic; Lingeman H. {Editeur Scientific}, Poppe H. {Editeur Scientific} {2005}. Validation of high performance liquid chromatography method for the determination of pancuronium in Pavulon injection. Journal of chromatography vol. 9459, no. 1-2, pp. 61.