Toxicological Analysis of Atracurium Besylate in Biological Materials by Using HPLC

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AIMS: Atracurium Besylate is a highly selective, competitive (non-depolarizing) neuromuscular blocking agent used in anesthesiology. Following intravenous administration atracurium is predominantly degraded, under physiological temperature and pH, by Hoffmann elimination to laudanosine. The aim of this study is to present a method for the toxicological analysis of atracurium in biological materials using HPLC.

METHODS: An isocratic reversed phase HPLC method coupled with UV detection has been developed for the determination of atracurium in human blood, urine, bile and tissues from the injection sites. The extraction technique used was simple and rapid; where blood, urine, bile and macerated injection site tissue samples were acidified with 0.5 M sulphuric acid to pH 4.2 and vortex mixed for 1 minute with acetonitrile. The samples were centrifuged; acetonitrile layer was separated and evaporated. A 20 µL aliquot of acetonitrile extract was injected onto the HPLC column. The extraction recovery was 79.6% at 50 µg/mL atracurium in blood.

The extracts were separated with a Lichospher Si-60 (150 mm x 4.6 mm I.D, 5 µm particle size) column. A mobile phase consisting of acetonitrile and 0.1 M dipotassium hydrogen ortho phosphate buffer (pH=5) in a ratio of 40:60 v/v was used at a flow rate of 1mL/min and UV detection was done at 280 nm.

RESULTS: Linear detector responses were observed for the calibration curve standards in the range of 0.2 - 100 µg/mL. The limit of detection (S/N ratio =3) and limit of quantitation (S/N ratio=10) for atracurium were 0.1µg/mL or 2 ng on column and 0.4 µg/mL or 8 ng on column respectively. The Relative Standard Deviation for atracurium besylate determination at 50 µg/mL was 2.2%.

The selectivity of the method was verified against endogenous compounds due to the matrices for which blank blood and urine samples extracted separately in the same way was injected and checked for the absence of interfering compounds.

CASE REPORT AND CONCLUSIONS: The method was validated and successfully applied in a case of fatality, where a 31 year old Sri Lankan male anesthetist, attached to a Government hospital was found unconscious in his duty room at night. An IV canula with a syringe was found in situ in one hand and an empty atracurium besylate vial was found at the scene. Medical investigations revealed that he was already dead and a postmortem was carried out by the Judicial Medical Officer. The samples from the body including blood, bile and tissues from the injection site together with the butterfly IV set with an empty injection syringe and empty atracurium vial recovered from the scene were sent to the Toxicology Section of the Government Analyst’s Department for examination. On analyzing the samples, 10.5 µg/mL, 28.8 µg/mL and 51.8 µg/mL atracurium besylate were identified in blood, bile and urine samples respectively. Further, atracurium besylate was identified in the empty vial, empty injection syringe recovered from the scene and the tissues taken from the injection site. This was the first recorded fatal case of atracurium besylate poisoning in Sri Lanka and possibly the first case of suicide in Sri Lanka involving atracurium besylate.

Keywords: Atracurium, HPLC, Fatality