

APPENDIX ONE

Housing of Animals

All the work involving the use of animals was approved by the Rhodes University animal ethics committee. The animals used throughout this study were male Wistar rats, that were purchased from the South African Institute for Medical Research (Johannesburg, South Africa), weighing 250-300g. The animals were chosen at random and assembled into groups of five. They were housed in opaque plastic cages with metal grid floors and covers, under a diurnal lighting cycle 12 light: 12 dark with food and water *ad libitum*. The animal room was windowless with an automatic temperature and lighting controls. Lights were turned on at 6am everyday. The intensity of the light illumination during the 12 hour light phase was approximately 300 μ Watts/cm². The temperature of the animal room was maintained between 20°C and 25°C while an extractor fan ensured the constant removal of stale air. The cages were cleaned daily.

APPENDIX TWO

Sacrificing and dissection of the animals

Rats were sacrificed swiftly by cervical dislocation and rapidly decapitated. To remove the brain, the top of the skull was removed and the brain was exposed by making an incision through the bone on either side of the parietal suture, from the foramen magnum to near the orbit. Using forceps the calvarium was lifted and removed, exposing the brain which was easily removed for use in experiments. All adhering tissue and visible traces of blood was eliminated by washing the tissue in 0.9% saline solution. The brains were either used immediately or stored at -70°C until needed.

Rat skin was removed by first, cutting away the hair with sharp scissors and then scraping it with a razor blade. A piece of skin from the back of the animal was then removed by making an incision on its back making certain not to pierce the flesh. The skins were removed and either used immediately or stored at -70°C until used. The skins were thawed and weighed at room temperature before use.

APPENDIX THREE

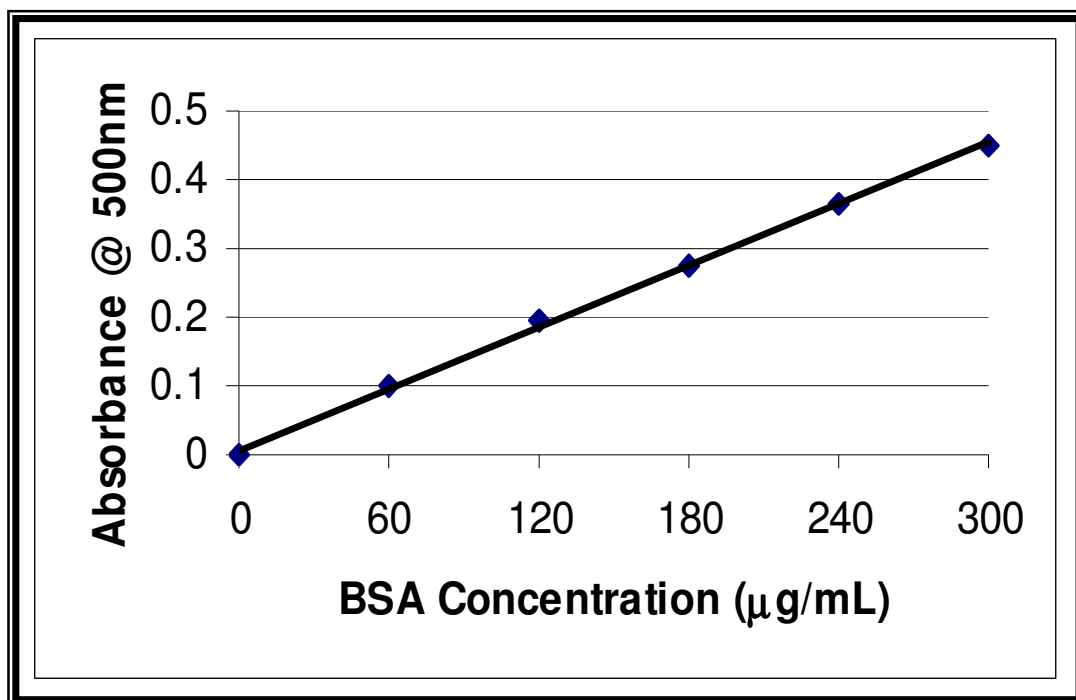
Protein Determination

Materials

Folin & Ciocalteu's reagent was purchased from Saarchem (PTY) Ltd, Krugersdorg, South Africa. The Bovine serum albumin (BSA) was supplied by Sigma Chemical CO, St. Louis, MO, USA. All other chemicals and reagents were obtained locally and were of the highest available purity.

Protein Determination

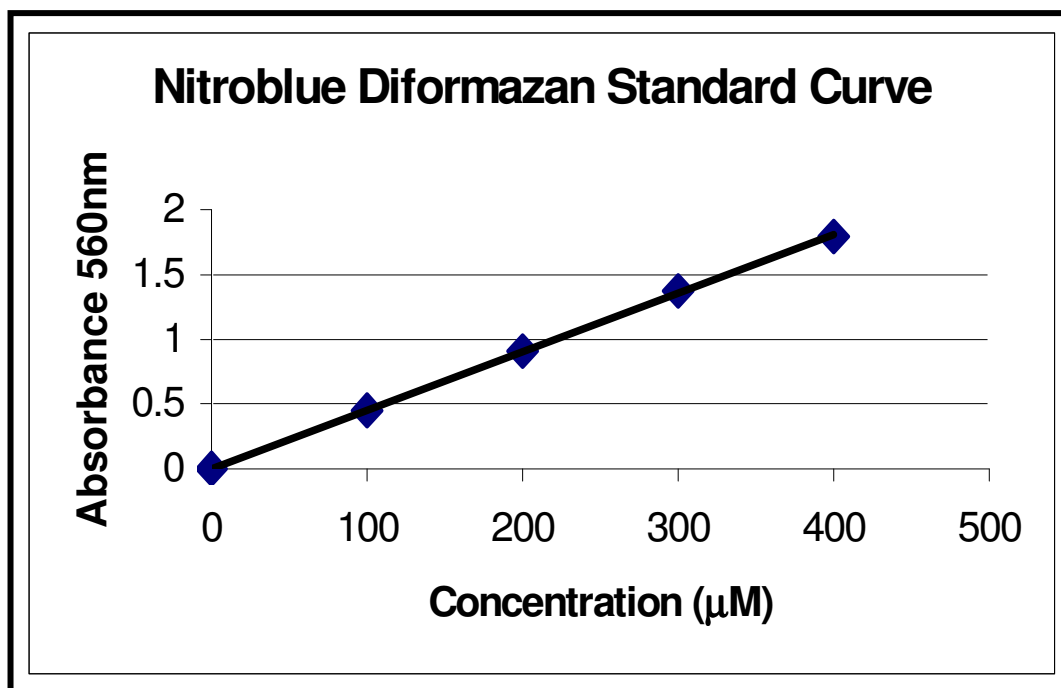
A modification of the method employed by Lowry et al (1950) was used throughout this study. 6mL of alkaline copper reagent (1mL 1% copper sulphate, 1mL 2% sodium tartrate, and 98mL 2% sodium carbonate) was added to 1mL homogenate in a set of clean test tubes. The tubes were mixed and allowed to stand at room temperature for ten minutes. Following this, 0.3mL of the Folin-Ciocalteu reagent was added to the tubes. The tubes were mixed and allowed to stand in the dark for 30 minutes at room temperature. After this period the absorbance was read at 500nm using a Shimadzu UV-160 A UV-visible spectrophotometer. A standard curve (0-300µg/mL) was also generated in the same manner, using 1mL BSA instead of homogenate.



Appendix 3: Protein Standard Curve Generated from BSA
($y = 0.0015x + 0.0072$, $r^2 = 0.999$)

APPENDIX FOUR

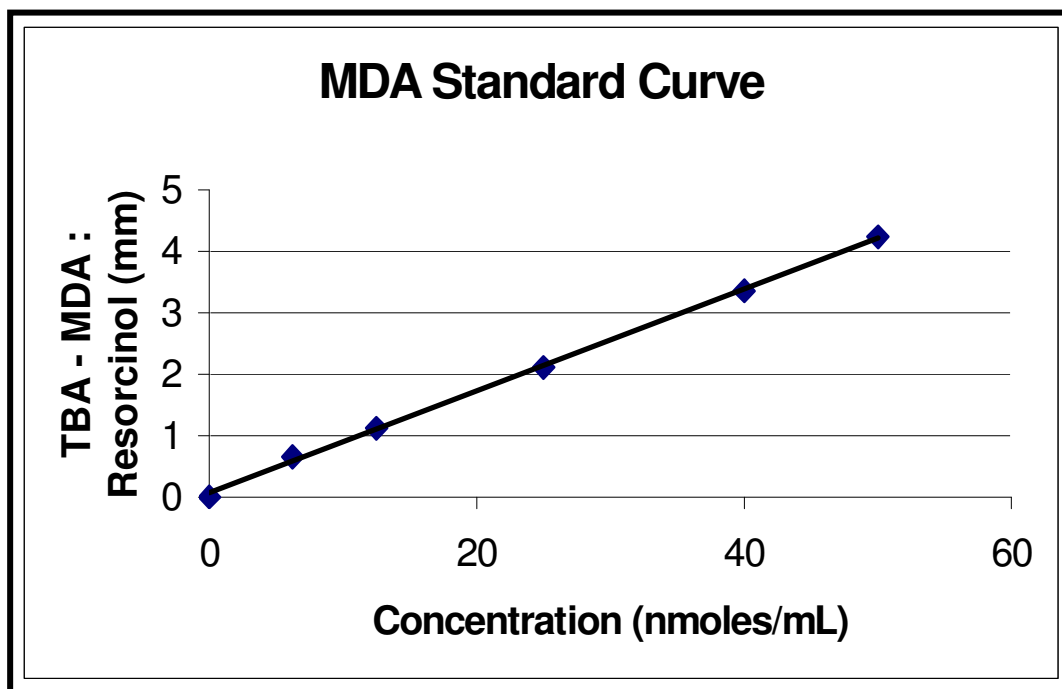
Nitroblue Diformazan Standard Curve



Appendix 4 : Nitroblue Diformazan Standard Curve
($y = 0.0045x + 0.0033$, $r^2 = 0.9998$)

APPENDIX FIVE

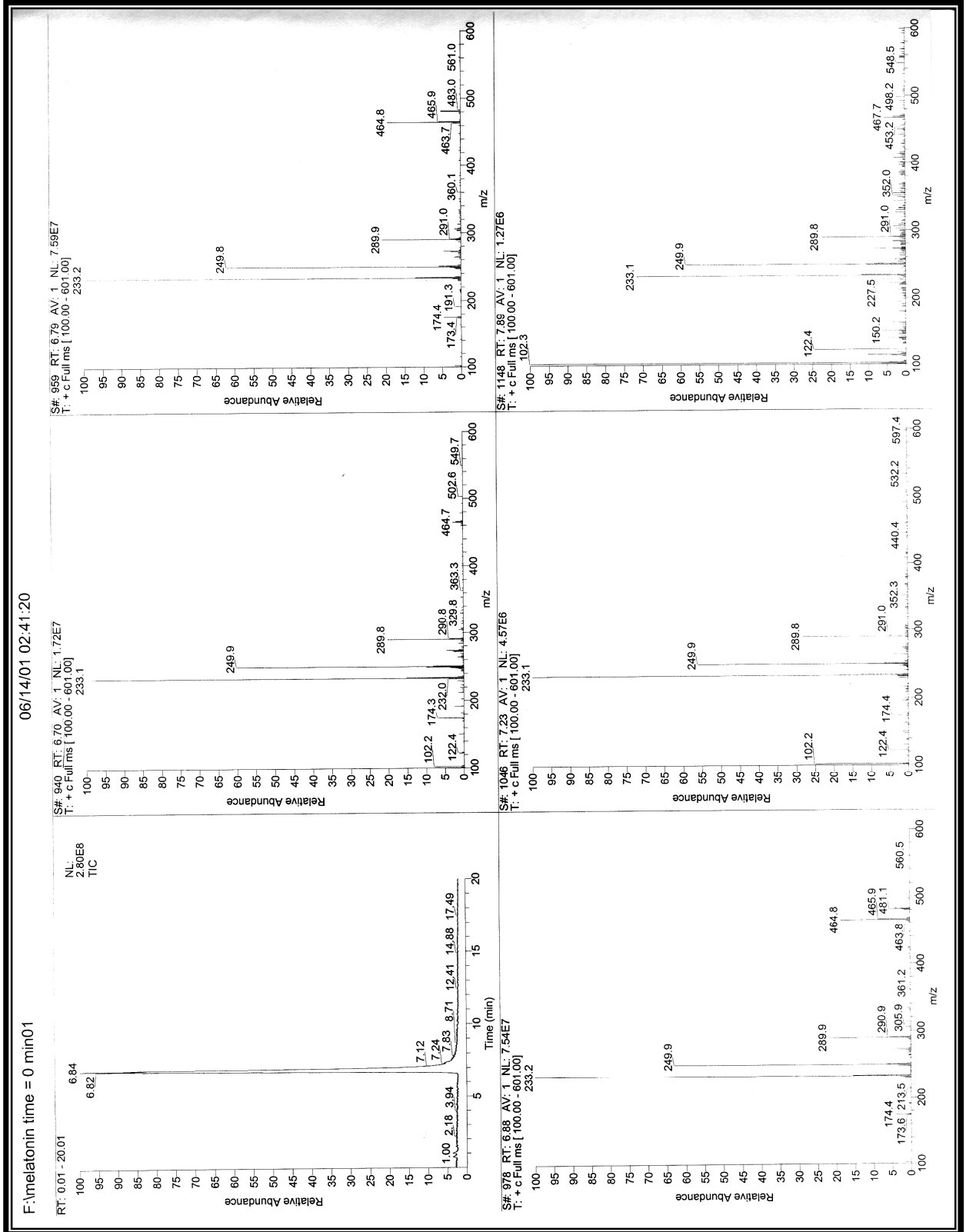
Lipid Peroxidation Standard Curve

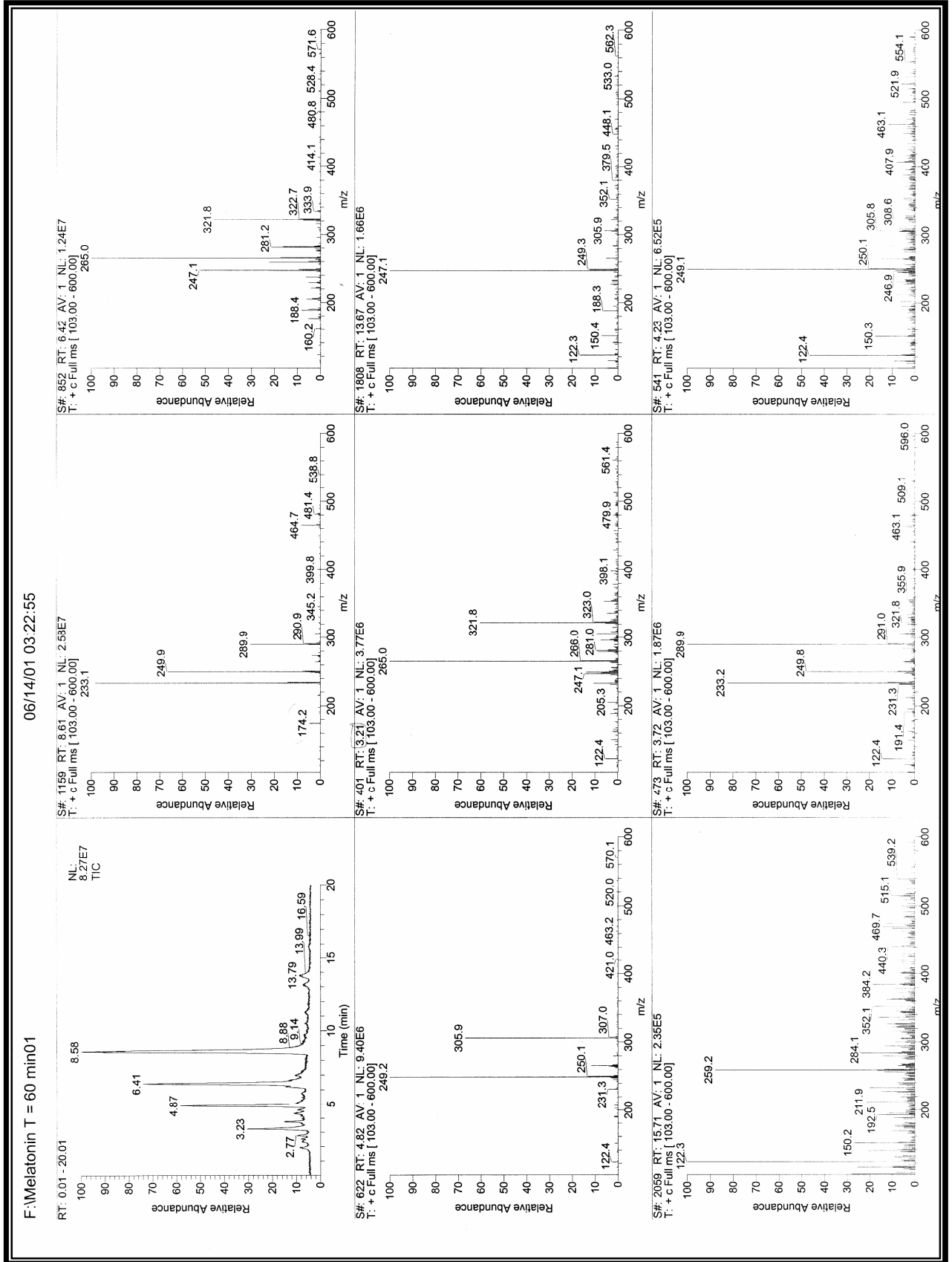


Appendix 5 : MDA (malondialdehyde) standard curve
($y = 0.0831x + 0.062$, $r^2 = 0.9991$)

APPENDIX SIX

LC-MS SCANS OF MELATONIN (PHOTODEGRADATION STUDY)





APPENDIX SEVEN

HPLC SCANS OF 6-HYDROCYMELATONIN AND Fe^{3+}

