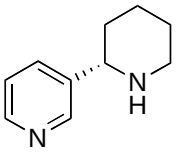
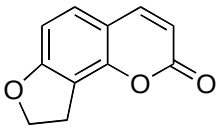
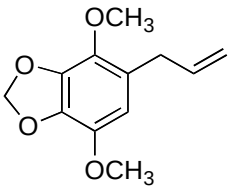
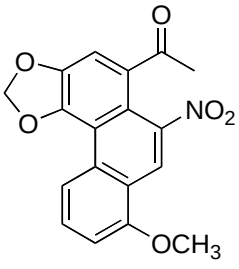
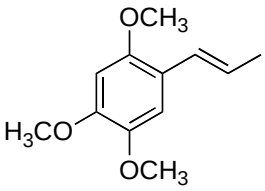
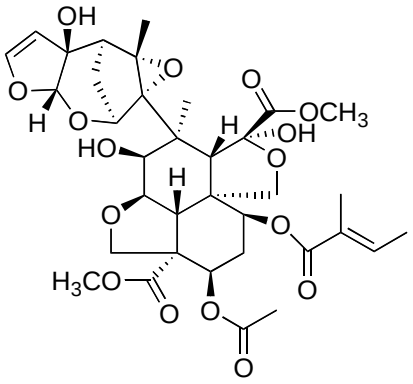
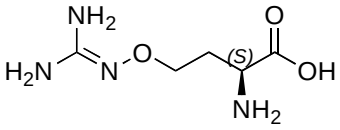
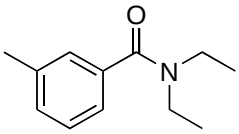
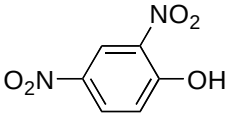
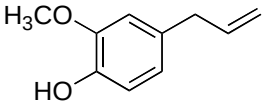
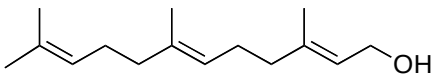
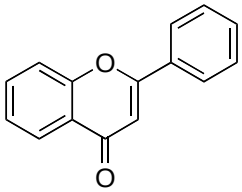
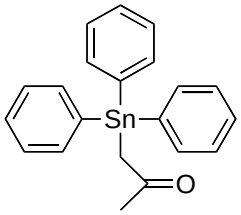
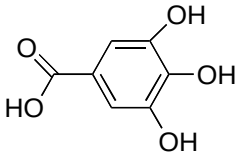
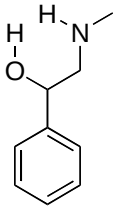
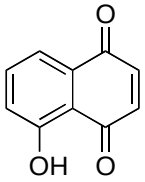
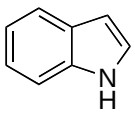
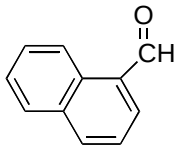
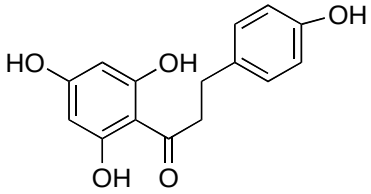
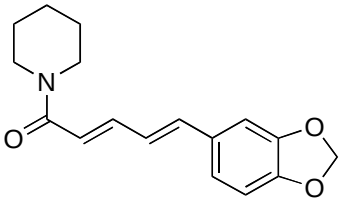
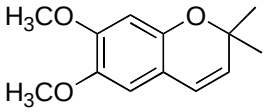
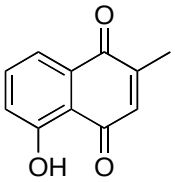
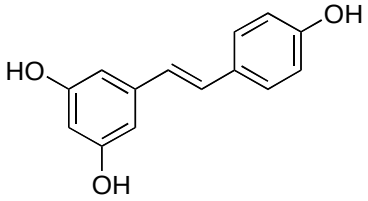
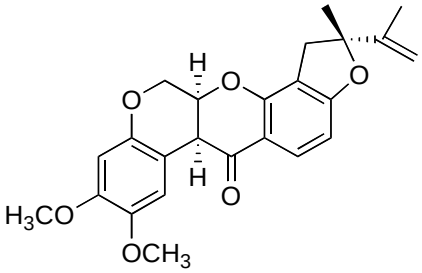
Supplemental tables

Table 1. Botanical pesticides tested for Nrf2/ARE induction.

Chemical	Class	Structure	ARE activation
Anabasine	Alkaloid		No
Angelicin	Coumarin		No
Apiole	Phenylpropanoid		No
Aristolochic acid	Alkaloid		No
Asarone	Phenolic ether		No

Azadirachtin	Terpenoid		No
Canavanine	Peptide		No
DEET	Amide		No
2,4-dinitrophenol	Phenol		No
Eugenol	Phenol		No
Farnesol	Sesquiterpene		No

Flavone	Flavonoid		No
Fentin acetate	Organotin		No
Gallic acid	Phenol		No
Halostachine	Alcohol		No
Juglone	Nafthoquinone		No
Indole	Alkaloid		No
1-naphthaldehyde	Aldehyde		No

Phloretin	Flavonoid		No
Piperine	Amide		No
Precocene	Benzopyran		No
Plumbagin	Napthoquinone		Yes
Resveratrol	Phenol		No
Rotenone	Flavonoid		No

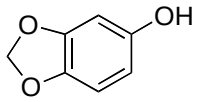
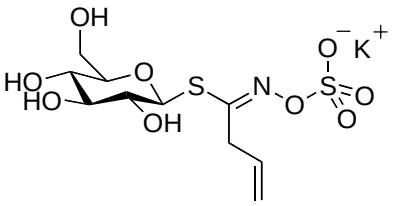
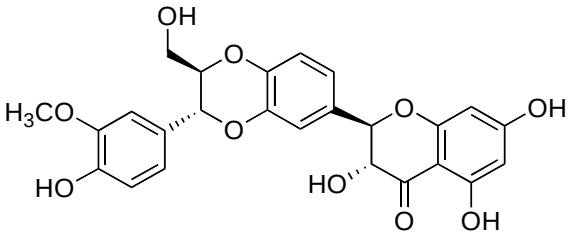
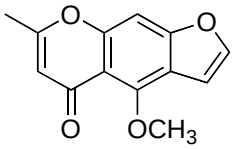
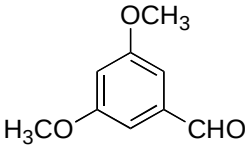
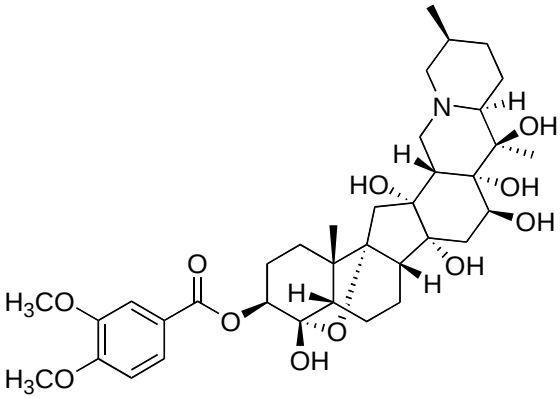
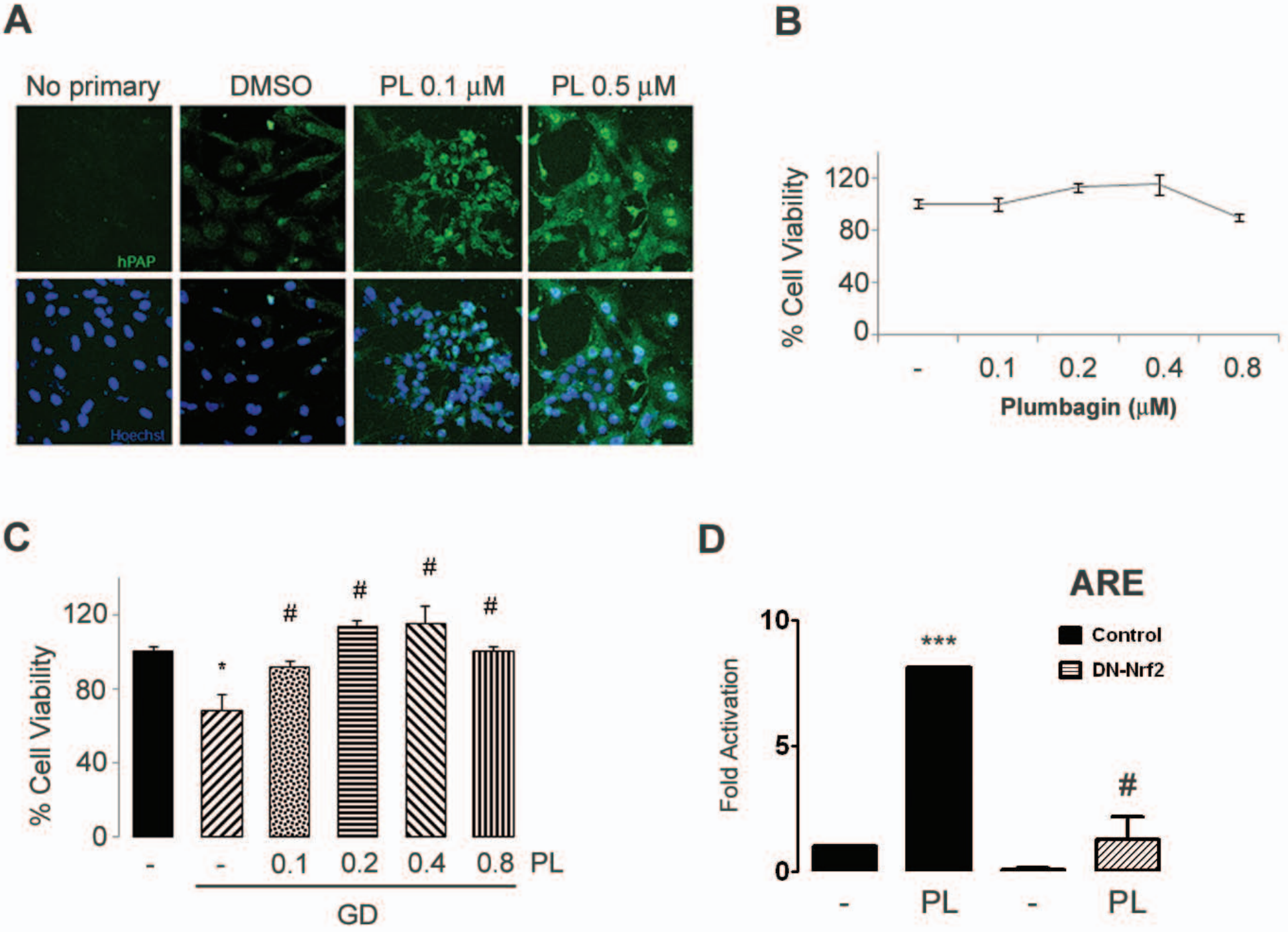
Sesamol	Phenol		No
Sinigrin	Glucoside		No
Sylimarin	Flavonoid		No
Visnagin	Phenolic ether		No
Veratraldehyde	Benzaldehyde		No
Veratrine	Alkaloid		No

Table 2. Cerebral blood flow in mice subjected to ischemia reperfusion. Cerebral blood flow was measured in vehicle and plumbagin (PL) treated animals at the indicated time points before and after middle cerebral artery occlusion (MCAO). Data are mean and S.D. (n=5-6). *p< 0.05 compared to vehicle I/R.

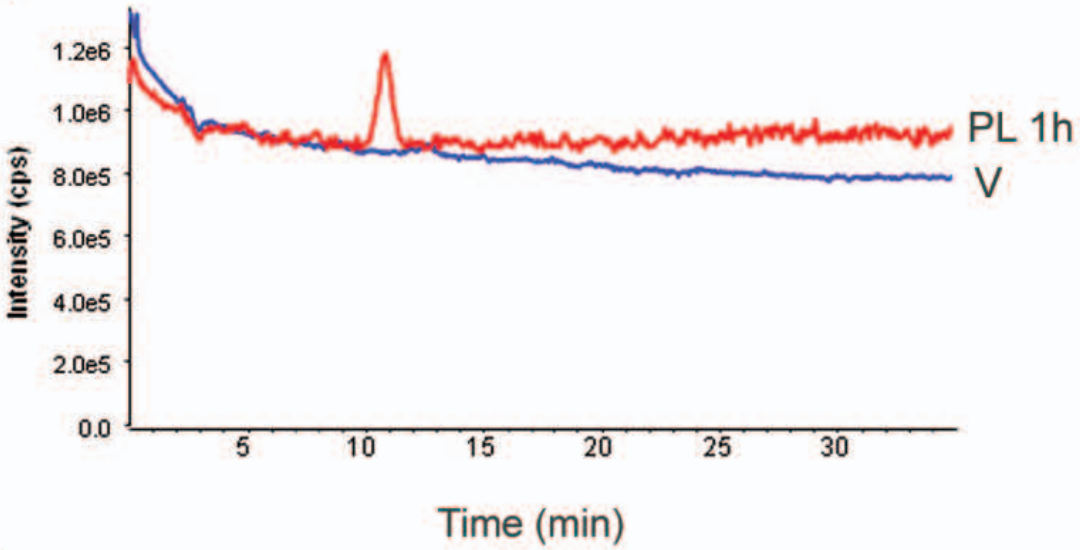
	Vehicle (%)	**PL (%)**
Pre-MCAO	100	100
MCAO 30 min	8.42 ± 3.1	10.7 ± 4.0
Post-MCAO 15 min	158 ± 30.0	167 ± 26.8
Post-MCAO 30 min	132 ± 15.6	148 ± 23.1
Post-MCAO 60 min	74.6 ± 20.9	77.3 ± 17.6
Post-MCAO 90 min	72.4 ± 14.1	69.5 ± 10.2
Post-MCAO 120 min	71.8 ± 14.8	88.6 ± 11.0
Post-MCAO 180 min	78.4 ± 14.0	95.0 ± 3.3*

Supplemental Figure 1. Plumbagin induces Nrf2-dependent genes in primary cultures. (A) Primary murine mixed (neurons and astrocytes) cultures were treated either with DMSO or the indicated concentrations of plumbagin. After 6 hrs the cells were fixed and processed for immunostaning using hPAP primary antibody, and Alexa Fluor 488-conjugated secondary antibody. Nuclei were counterstained with Hoechst. (B) Primary rat cortical neurons treated with vehicle (-) or the indicated concentrations of plumbagin for 24 hrs were assessed for viability by MTT assay. Data are presented as mean and S.D. (n=3). (C) Neuronal cultures treated with vehicle (-) or the indicated concentrations of plumbagin (PL) were subjected to glucose deprivation (GD) and cell viability assessed after 24 hrs. Date are mean and S.D. (n=3). *, $p<0.001$ compared to control; #, $p<0.01$ compared to GD. (D) SH-SY5Y cells were transfected with ARE- firefly luciferase reporter and pRL-TK control vector, and either pEF (Control) or dominant negative Nrf2 (DN-Nrf2) constructs. 24 hrs post-transfection cells were treated with either vehicle (-) or 1 µM plumbagin (PL). The relative light intensities were normalized, and signal averages were determined. Data are mean and S.D. (n=4-6). ***, $p=0.0019$ compared to vehicle; #, $p=0.0018$ compared to PL.

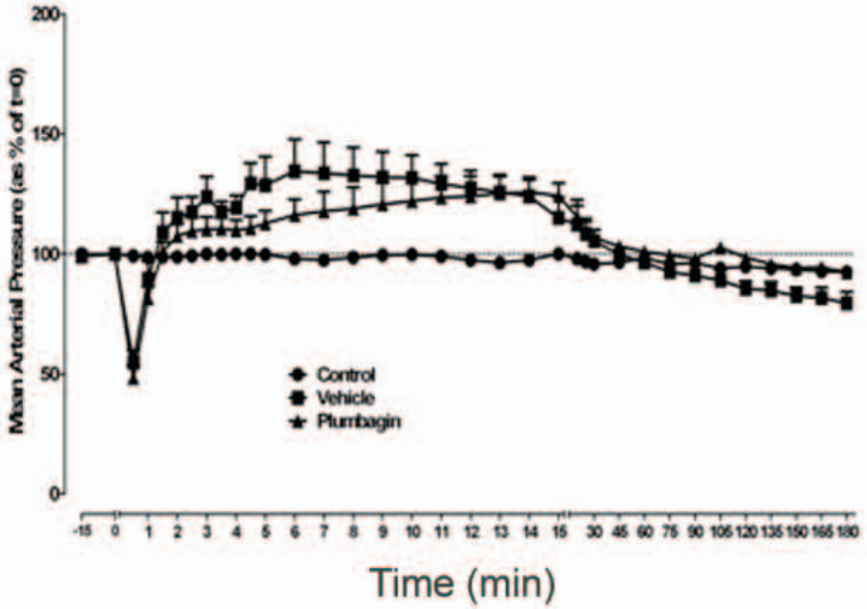
Supplemental Figure 2. Plumbagin cerebral bioavalability and effects on mean arterial pressure and heart rate. (A) Representative HPLC/ tandem mass spec chromatogram identifying plumbagin (PL) in brain extracts 1 hr post administration. Mean arterial pressure (B) and heart rate (C) were measured in vehicle and plumbagin treated animals pre- and post-occlusion as described. Data are mean and S.D. (n=5-6).


A
No primary
DMSO
PL 0.1 μM
PL 0.5 μM
hPAP
Hoechst
B
% Cell Viability
120
80
40
0
-
0.1
0.2
0.4
0.8
Plumbagin (μM)
C
% Cell Viability
120
80
40
0
*
#
#
#
#
-
-
0.1
0.2
0.4
0.8
PL
GD
D
ARE
Control
DN-Nrf2
Fold Activation
10
5
0
***
#
-
PL
-
PL

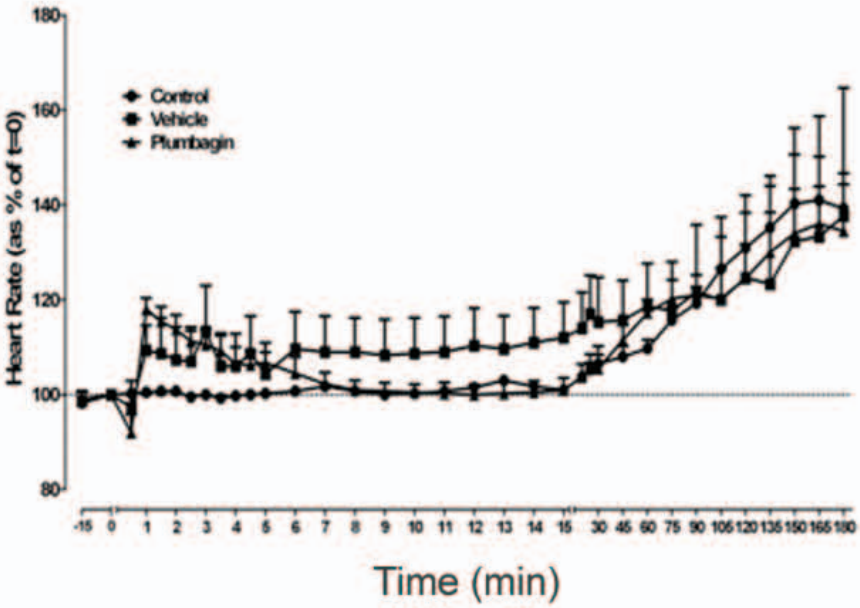
A


Intensity (cps)
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
0.0
5
10
15
20
25
30
PL 1h
V
Time (min)

B


Mean Arterial Pressure (as % of t=0)
200
150
100
50
0
Control
Vehicle
Plumbagin
-15 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)

C


Heart Rate (as % of t=0)
180
160
140
120
100
80
Control
Vehicle
Plumbagin
-15 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)