

# Mechanisms of Herb-Drug Interactions Involving Cinnamon and Cytochrome P450 2A6: Focus on Time-dependent Inhibition by Cinnamaldehyde and 2-Methoxycinnamaldehyde

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## Supplemental Material 5 LCMS Heme Analysis Conditions

### LC/MS Conditions for Heme Adduct Method

The LC/MS system was composed of an UHPLC (Waters I Class, Milford, MA) and a hybrid quadrupole/time of flight mass spectrometer (AB SCIEX 5600 TripleTOF, Applied Biosystems, Framingham, MA). Data were collected by Analyst TF (version 1.7.1, Applied Biosystems, Framingham, MA), and were analyzed with PeakView (version 2.2, Applied Biosystems) and Metabolite Pilot (version 2.0.2, Applied Biosystems). The details were as follows:

#### LC Conditions

Column:	Agilent Waters XDB C <sub>18</sub> 2.1 × 50 mm, 5 μ (Santa Clara, CA)
Column Temperature:	Ambient
Needle Wash:	10% ammonium hydroxide, 40% water, 50% isopropyl alcohol
Mobile Phases:	(A) 0.1% ammonium hydroxide in water (B) 0.1% ammonium hydroxide in isopropyl alcohol
Gradient:	(B) 0% (0 - 1 min), 100% (14-15 min), 0% (15.1-20 min)
Flow Rate:	500 μL/min
Sample Tray Temperature:	Room temperature
Sample Injection Volume:	10 μL

#### MS Conditions

Ionization Mode	Positive
Curtain Gas:	35
Temperature:	600°C
GAS1:	60
GAS2:	60
ISVF:	4500 V
Survey Scan Settings:	
Scan Range:	100-2000 m/z
Accumulation Time:	0.1 sec
DP:	100
CE:	5
CES:	0
IDA Settings:	
Maximum Scans per cycle:	4 (Product Ion)
Scan Range:	100-2000 m/z
Accumulation Time:	0.1 sec
DP:	100
CE:	35

CES: 20  
IDA Trigger Type: Mass Defect Filter and Largest Peak  
Mass Defect Filters: 70 mDa error/400 Da window with  $C_{34}H_{34}N_4O_4$  + Compound  
Formula and  $C_{34}H_{32}N_4O_4Fe$  + Compound Formula  
Exclude previous ions: 1 sec  
Minimum CPS for trigger: 200