

Cumin Prevents 17 β -Estradiol-Associated Breast Cancer in ACI Rats

Farrukh Aqil ^{1,2}, Jeyaprakash Jeyabalan ¹, Radha Munagala ^{1,2}, Iqbal Ahmad ³, David J. Schultz ⁴ and Ramesh C. Gupta ^{1,5,*}

¹ James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA; farrukh.aqil@louisville.edu (F.A.); jp3pbiotech@gmail.com (J.J.); radha.munagala@gmail.com (R.M.)

² Department of Medicine, University of Louisville, Louisville, KY 40202, USA

³ Department of Ag Microbiology, Aligarh Muslim University, Aligarh 202002, India; iqbalahmad8@yahoo.co.in

⁴ Department of Biology, University of Louisville, Louisville, KY 40208, USA; david.schultz@louisville.edu

⁵ Department of Pharmacology and Toxicology, 580 S. Preston St, Rm 304E, Baxter II Research building, University of Louisville, Louisville, KY 40202, USA

* Correspondence: rcgupta@louisville.edu; Tel.: +502-852-3684; Fax: +502-852-3842

Supplementary Table S1. Detailed formula of the AIN93M purified diet (Catalog # TD.94048; Harlan Laboratories, Madison, WI)

Teklad Custom Research Diet Data Sheet

TD.94048 AIN-93M Purified Diet

| Formula | g/Kg |
|---------------------------------|-------------|
| Casein | 140.0 |
| L-Cystine | 1.8 |
| Corn Starch | 465.692 |
| Maltodextrin | 155.0 |
| Sucrose | 100.0 |
| Soybean Oil | 40.0 |
| Cellulose | 50.0 |
| Mineral Mix, AIN-93M-MX (94049) | 35.0 |
| Vitamin Mix, AIN-93-VX (94047) | 10.0 |
| Choline Bitartrate | 2.5 |
| TBHQ, antioxidant | 0.008 |

Footnote

Reference: J. Nutr. 123:1939-1951, 1993. Formulated for the maintenance of adult, non-reproducing rodents.

Selected Nutrient Information¹

| | % by weight | % kcal from |
|---------------------|--------------------|--------------------|
| Protein | 12.4 | 13.8 |
| Carbohydrate | 68.4 | 76.0 |
| Fat | 4.1 | 10.2 |
| Kcal/g | 3.6 | |

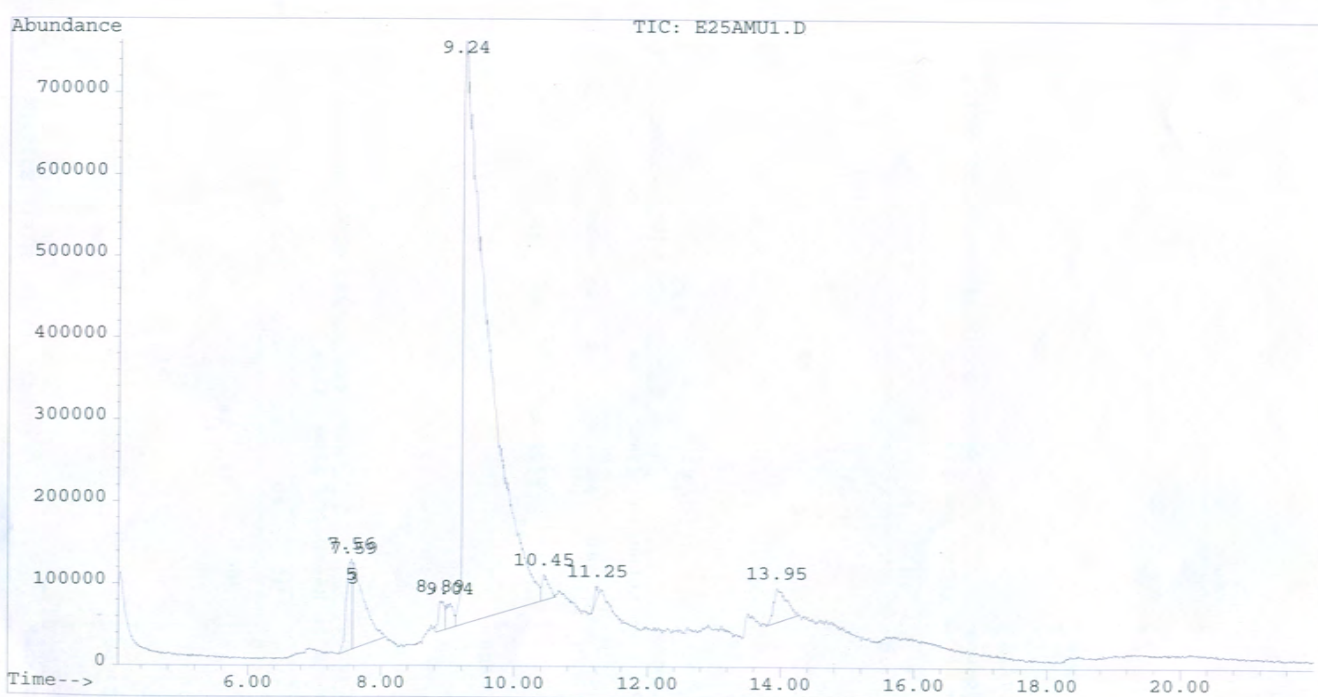
¹ Values are calculated from ingredient analysis or manufacturer data

Teklad Diets are designed & manufactured for research purposes only.

Supplementary Table S2 . GC-MS Analysis - Graphics Report

Graphics Report

File : C:\HPCHEM\1\E25AMU1.D
Operator : SVKL
Acquired : 20 May 110 9:28 am using AcqMethod MANALI
Sample Name: Cumin MeOH Ext. 15/04/10 FH 110
Misc Info : 100-10-250- 3M-30-280-5M-MeIOH-HP5
Vial Number: 1

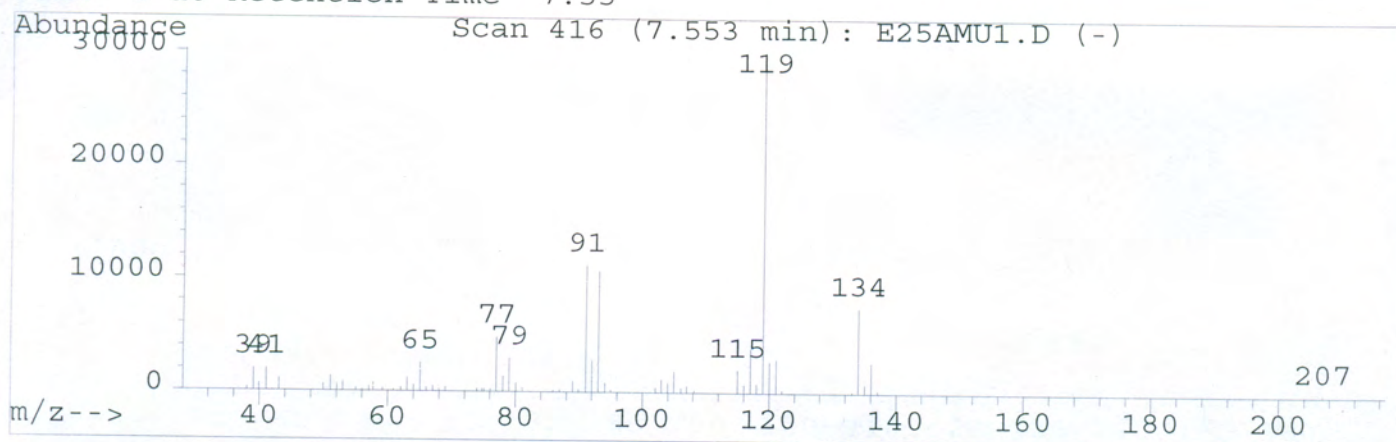


C:\HPCHEM\1\E25AMU1.D

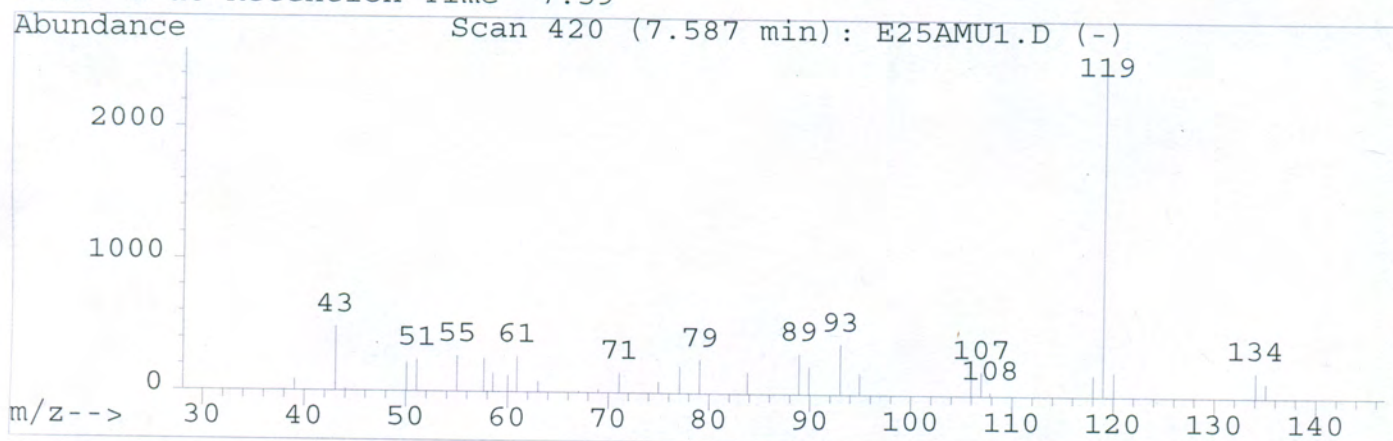
Thu May 20 11:10:02 2010

Page 1 of 3

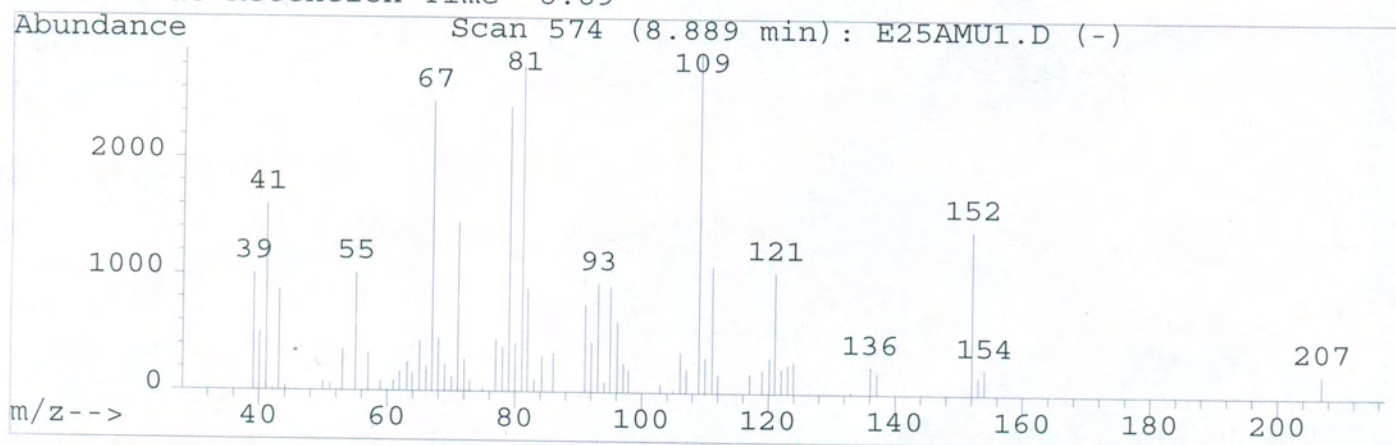
Peak 1 at Retention Time 7.55



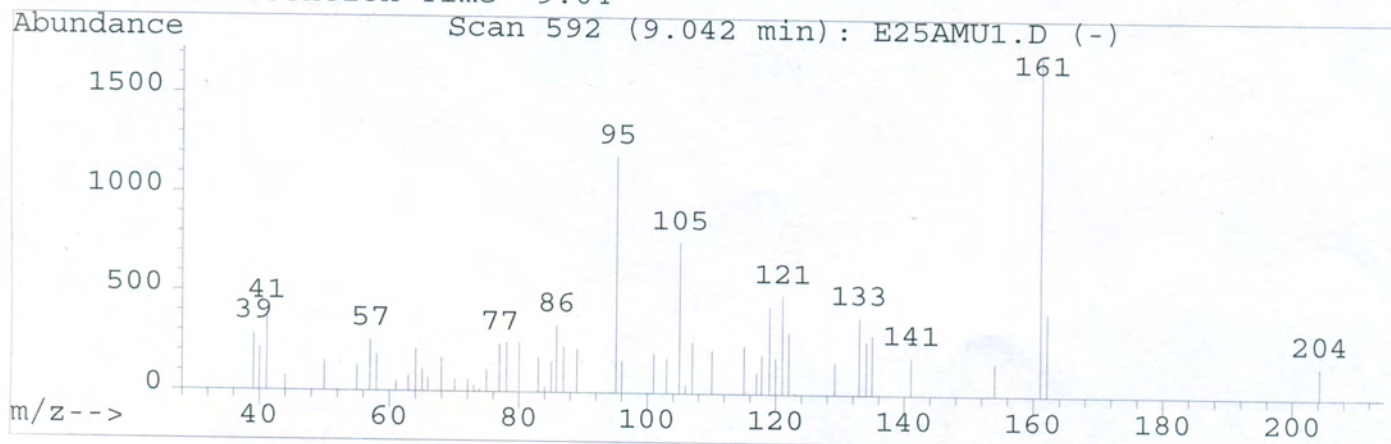
Peak 2 at Retention Time 7.59



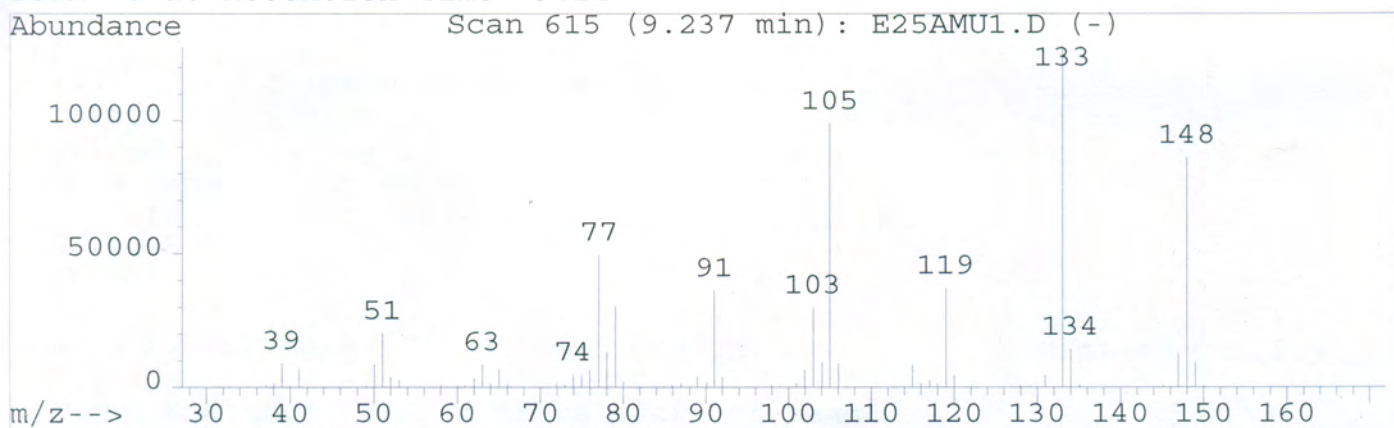
Peak 3 at Retention Time 8.89



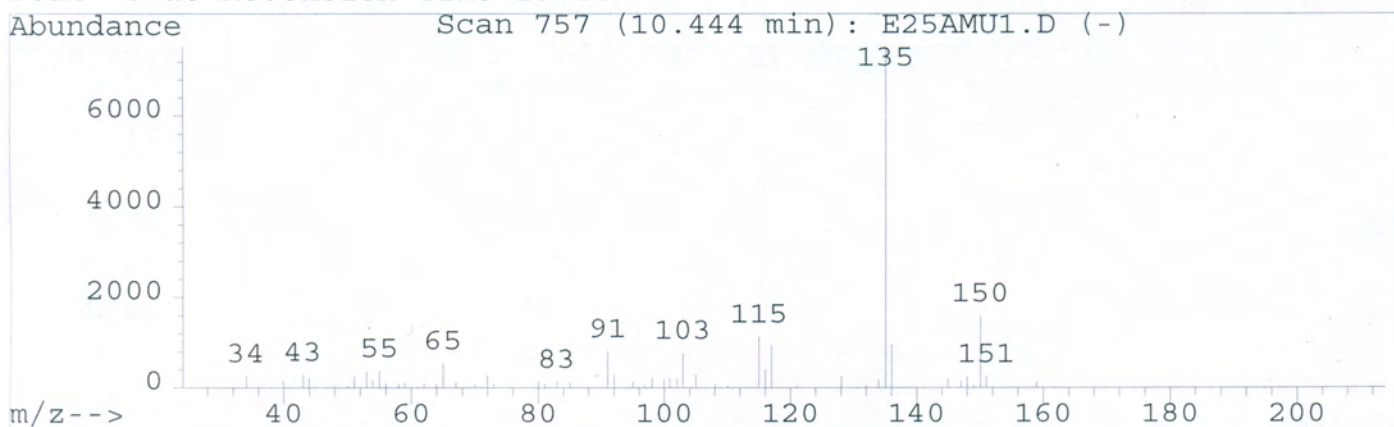
Peak 4 at Retention Time 9.04



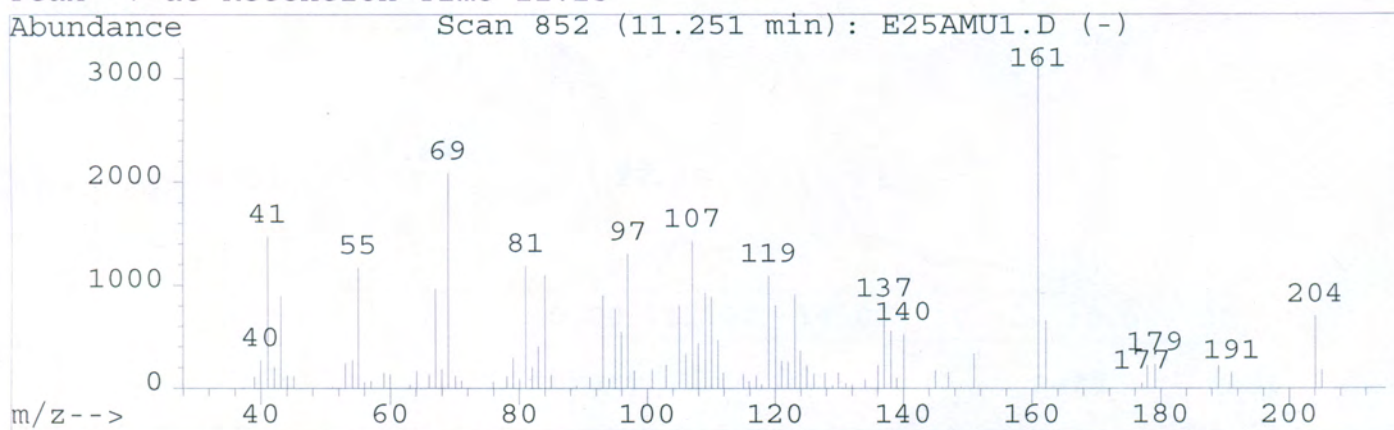
Peak 5 at Retention Time 9.24



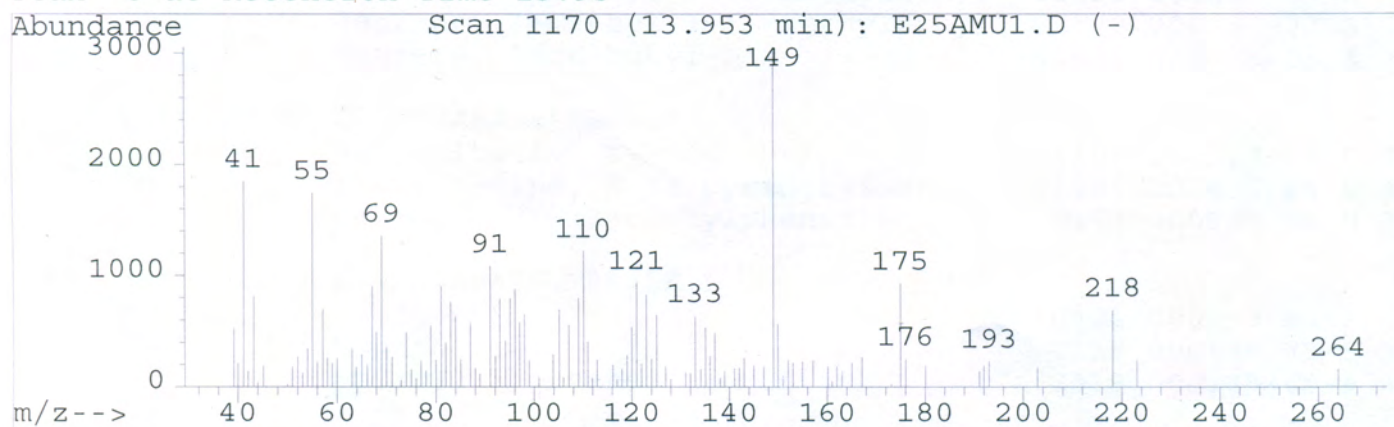
Peak 6 at Retention Time 10.44



Peak 7 at Retention Time 11.25



Peak 8 at Retention Time 13.95



Supplementary Table S3 . Library Research Report

Summary Library Search Report

Information from Data File:

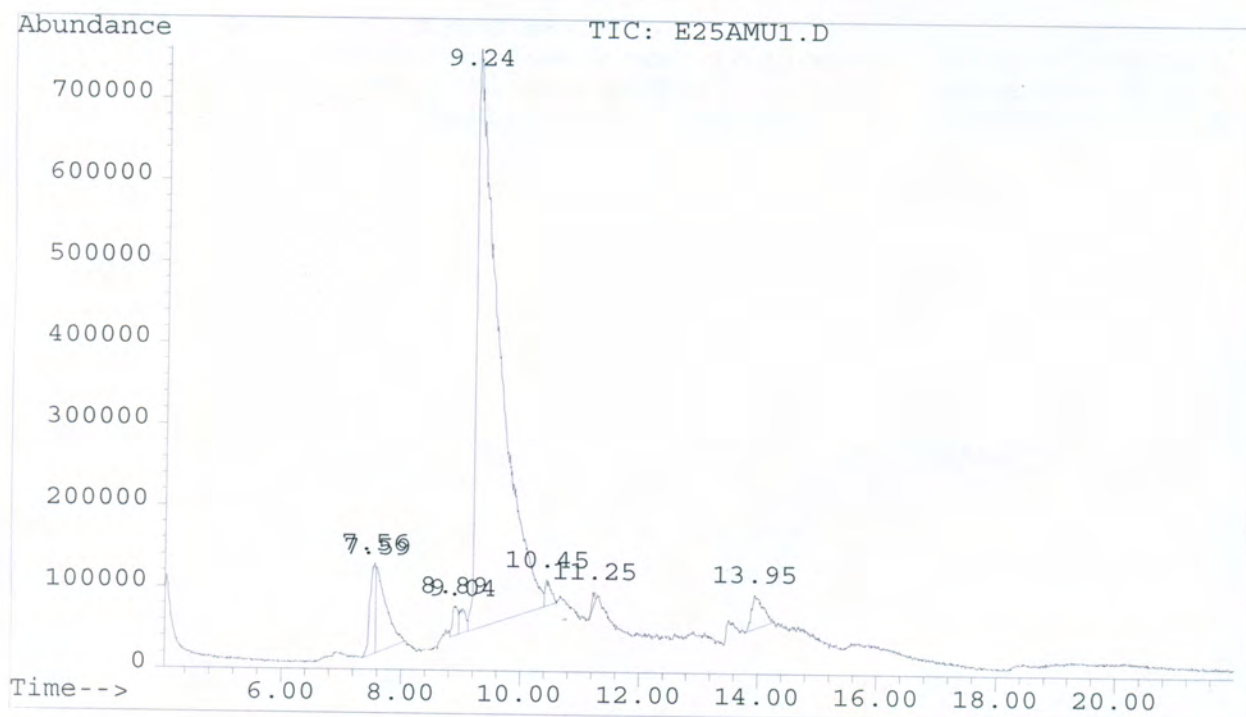
File : C:\HPCHEM\1\E25AMU1.D
 Operator : SVKL
 Acquired : 20 May 110 9:28 am using AcqMethod MANALI
 Sample Name: Cumin MeOH Ext. 15/04/10 FH 110
 Misc Info : 100-10-250- 3M-30-280-5M-MeIOH-HP5
 Vial Number: 1

Search Libraries: C:\DATABASE\nbs75k.1

Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak

Integration Params: AutoIntegrate



| PK# | RT | Area% | Library/ID | Ref# | CAS# | Qual |
|-----|------|-------|------------------------------------|-------|-------------|------|
| 1 | 7.55 | 3.07 | C:\DATABASE\NBS75K.L | | | |
| | | | Benzene, 1-methyl-4-(1-methylethyl | 65539 | 000099-87-6 | 92 |
| | | | Benzene, 1-methyl-3-(1-methylethyl | 65579 | 000535-77-3 | 70 |
| | | | Benzene, tert-butyl- | 65529 | 000098-06-6 | 70 |
| 2 | 7.59 | 5.99 | C:\DATABASE\NBS75K.L | | | |
| | | | Benzonitrile, 4-hydroxy- | 64499 | 000767-00-0 | 47 |
| | | | 1-Butanamine, N-(2-pyridinylmethyl | 12959 | 007032-24-8 | 40 |
| | | | Ethanone, 1-(3-methylphenyl)- | 6153 | 000585-74-0 | 38 |
| 3 | 8.89 | 1.04 | C:\DATABASE\NBS75K.L | | | |
| | | | Pulegone | 10406 | 000089-82-7 | 46 |
| | | | Mequinol | 4138 | 000150-76-5 | 30 |
| | | | Spiro[2.5]octane | 2339 | 000185-65-9 | 30 |
| 4 | 9.04 | 1.03 | C:\DATABASE\NBS75K.L | | | |
| | | | 1H-Cyclopropa[a]naphthalene, 1a,2, | 23897 | 017334-55-3 | 47 |
| | | | 3a,7-Methano-3aH-cyclopentacyclooc | 23952 | 000469-92-1 | 43 |
| | | | 2-Hexen-1-one, 1-(2-hydroxy-5-meth | 23838 | 051956-79-7 | 35 |

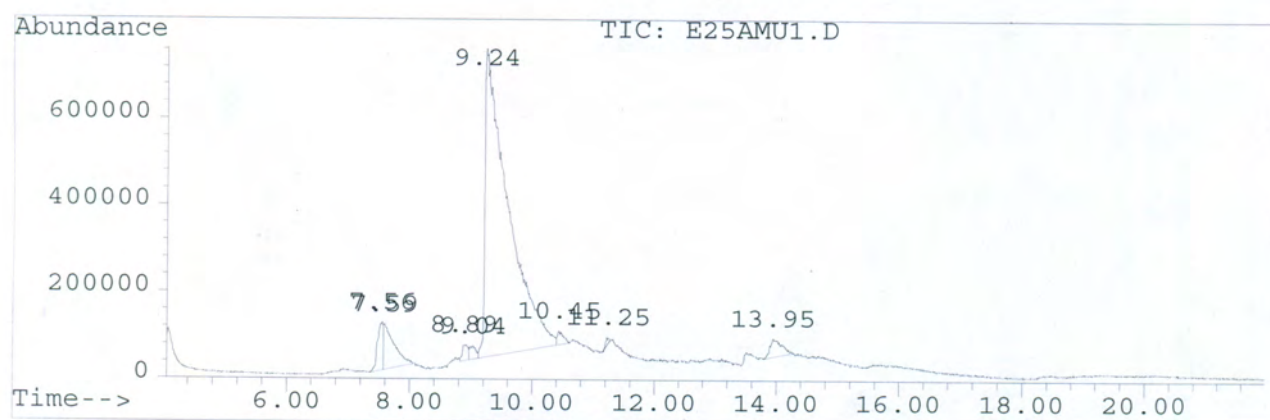
| Pk# | RT | Area% | Library/ID | Ref# | CAS# | Qual |
|-----|-------|-------|------------------------------------|-------|-------------|------|
| 5 | 9.24 | 85.11 | C:\DATABASE\NBS75K.L | | | |
| | | | Benzaldehyde, 4-(1-methylethyl)- | 66596 | 000122-03-2 | 97 |
| | | | Ethanone, 1-(2,4-dimethylphenyl)- | 66595 | 000089-74-7 | 91 |
| | | | Ethanone, 1-(2,5-dimethylphenyl)- | 9319 | 002142-73-6 | 87 |
| 6 | 10.44 | 1.03 | C:\DATABASE\NBS75K.L | | | |
| | | | Thymol | 66816 | 000089-83-8 | 74 |
| | | | Phenol, 2-methyl-5-(1-methylethyl) | 66779 | 000499-75-2 | 72 |
| | | | Phenol, p-tert-butyl- | 9864 | 000098-54-4 | 58 |
| 7 | 11.25 | 0.24 | C:\DATABASE\NBS75K.L | | | |
| | | | Carotol | 28216 | 000465-28-1 | 53 |
| | | | Naphthalene, 1,2,3,4,4a,5,6,8a-oct | 23957 | 039029-41-9 | 50 |
| | | | Germacrene D | 23911 | 023986-74-5 | 49 |
| 8 | 13.95 | 2.51 | C:\DATABASE\NBS75K.L | | | |
| | | | Trichothec-9-en-8-one, 12,13-epoxy | 36571 | 002199-06-6 | 32 |
| | | | Silane, chlorotripropyl- | 20645 | 000995-25-5 | 27 |
| | | | 2(1H)-Naphthalenone, octahydro-1,4 | 17896 | 022738-31-4 | 25 |

Supplementary Table S4 . Area Percent Report

Area Percent Report -- Sorted by Signal

Information from Data File:

File : C:\HPCHEM\1\E25AMU1.D
 Operator : SVKL
 Acquired : 20 May 110 9:28 am using AcqMethod MANALI
 Sample Name: Cumin MeOH Ext. 15/04/10 FH 110
 Misc Info : 100-10-250- 3M-30-280-5M-MeIOH-HP5
 Vial Number: 1
 CurrentMeth: C:\HPCHEM\1\METHODS\MANALI.M



| Retention Time | Area | Area % | Ratio % | Type | Width |
|------------------------|-----------|--------|---------|------|-------|
| Total Ion Chromatogram | | | | | |
| 7.556 | 6641441 | 3.065 | 3.602 | PV | 0.101 |
| 7.587 | 12979705 | 5.991 | 7.039 | VV | 0.207 |
| 8.891 | 2244180 | 1.036 | 1.217 | VV | 0.082 |
| 9.041 | 2223002 | 1.026 | 1.206 | VV | 0.125 |
| 9.242 | 184390447 | 85.106 | 100.000 | VV | 0.321 |
| 10.446 | 2222522 | 1.026 | 1.205 | VV | 0.106 |
| 11.253 | 520542 | 0.240 | 0.282 | BV | 0.055 |
| 13.951 | 5437390 | 2.510 | 2.949 | PB | 0.170 |

Supplementary Table S5. Allometric dose calculations based on body surface area

Average feed intake/rat/day = 10 g; Cumin extract dose/rat/day @1% (w/w) supplement = 100 mg; Average body weight of female ACI rat = 200 g (0.2 kg); Intake of dried extract diet (mg/kg body weight) @ 1% = 100 mg/0.2 kg = 500 mg

$$\begin{aligned}\text{Human Eq. Dose (mg/kg)} &= \text{Animal dose (mg/kg)} \times \frac{\text{Animal Km (6 for rats)}}{\text{Human Km (37 for 60 kg adult)}} \\ &= 500 \times \frac{6}{37} \\ &= 81 \text{ mg (equivalent of 0.63 g of cumin seeds)}\end{aligned}$$

where *Km factor* is the body weight (kg) divided by body surface area (m²)

Reference: Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22:659–661