

## COMPARATIVE CHEMICAL STANDARDIZATION STUDIES ON *EMBLICA OFFICINALIS* GAERTN. FRUITS COLLECTED FROM TWO DIFFERENT LOCALITIES

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### ABSTRACT

In the present study, fruits of *Emblica officinalis* Gaertn. collected from Thanjavur and Trichy were analyzed from chemical standardization point of view. From the studies it is concluded that Trichy sample is better as the contents of flavones, alkaloids and tannins were more in these samples. But gallic acid percentage is more in Thanjavur sample from the purity point of view. It was concluded that environmental contamination and storage play considerable role in deciding the chemical potentials.

**Keywords:** *Emblica officinalis* Gaertn. flavones, Alkaloids

### INTRODUCTION

After post General agreement on tariffs and trade (GATT) era there is big resurgence in herbal based medicines and researches are focusing towards finding out their potentials & controlling various diseases. For preparation of any herbal formulation identification, evaluation and standardization is rudimentary [1]. Utilization of herbal supplements has increased significantly over the past 30 years. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act (DSHEA) of 1994. Research Institutes has isolated marker compounds from important Indian medicinal plants, characterized them and documented their utility in standardizing the plant extracts.

In the present study Amla botanically equated as *Emblica officinalis* (Euphorbiaceae) Gaertn. Occupied a hallowed position in Ayurveda- an Indian indigenous system of medicine (EO) is studied from chemical standardisation point of view. It is also equated to *Phyllanthus Emblica* or Indian gooseberry. *Emblica officinalis* Gaertn. a small or medium - sized deciduous tree with smooth, greenish gray, exfoliating bark. Feathery leaves with small narrowly oblong, pinnately arranged leaflets. Fruits depressed globose, ½ -1 inch in dia, fleshy and obscurely 6-lobed, containing 6 trigonous seeds. The fruit pulp contains moisture, 81.2; Protein, 0.5; Fat, 0.1; Mineral matter, 0.7; Fibre, 3.4; Carbohydrates, 14.1; Ca, 0.05; and Vitamin C 600 mg/ 100g [2].

A tannin containing gallic acid, ellagic acid and glucose are present in the fruit which prevents or retards the oxidation of the vitamin and renders the fruit a valuable antiscorbutic agent.

Amla is acrid, cooling, refrigerant, diuretic and laxative. The raw fruit is eaten as an aperient. Dried fruit is useful in haemorrhage, diabetes [3], ulcer [4], diarrhea and in dysentery. In combination with Iron, it is used as a remedy for anaemia [5], Jaundice and dyspepsia. A fermented liquor prepared from the fruit is used in Jaundice [6], dyspepsia and cough. 'Triphala' [7], consisting of equal parts of powdered *Emblic myrobalan*, *Belleric myrobalan* and *Chebulic myrobalan* is used as a laxative and in headache, biliousness, dyspepsia; constipation, piles enlarged liver and ascites. The exudation from incisions on the fruit is used as an external application for inflammation of the eye [8]. The flowers are cooling, refrigerant and aperient. The root and bark are astringent. Present study is taken up to standardize the genuine source of such as a potential drug which could be of immense use in increasing the immunity as well life span of the human society.

### MATERIALS AND METHODS

#### Collection of plant material

Fruits of *Emblica officinalis* Gaertn. were purchased from local markets in Trichy and Thanjavur in the month of January 2012. Identification and authentication were done in the Department

CARISM, SASTRA University, Thanjavur. The collected materials were cleaned, dried and coarsely powdered. These powdered materials were used for physicochemical, phytochemical and fluorescence analysis. The procedure recommended by India pharmacopoeia was followed.

### RESULTS AND DISCUSSION

Data obtained revealed interesting chemical features. Table 1 shows the extraction yield of herbal extracts obtained in Thanjavur and Trichy samples. Among the solvents used for extraction alcohol was found to have maximum extractive yield followed by chloroform and hexane. (Thanjavur). In Trichy samples, hexane extractive was found to be more followed by alcohol and chloroform extracts. Extractive values in samples will depict their rich bioactive potentials [9].

Loss on drying, ash value, acid insoluble, water soluble and sulphated ash value of the two plant extracts in different regions (Thanjavur and Trichy), are given in Table 2. Thanjavur samples proved to be better than Trichy samples. Tests of purity revealed that the samples procured from both the places were pure.

Preliminary phytochemical analysis of *Emblica officinalis* Gaertn. extracts of both the extracts showed the presence of phenol, reducing sugar, carbohydrate, flavones, glycosides, saponins, alkaloids, quinone and tannins. Steroids, amino acids and protein were absent in (Table 3).

Table 4 gives the data on fluorescence analysis of Thanjavur and Trichy plant drug samples. In Thanjavur samples powder treated with hydrochloric acid and picric acid appeared darker in 254 nm than 366 nm. In Trichy samples powder treated with Nitric acid, Hydrochloric acid and Picric acid appeared darker in 254 nm than 366 nm.

From XRF data, present in Table 6 revealed the potassium percentage to be more in Thanjavur. Iron, silica and lead content were significantly higher in samples procured from Trichy depicting the sample is inorganically rich but loading with heavy metal contamination.

HPTLC data, percentage of gallic acid in *Emblica officinalis* Gaertn. (Thanjavur) was found to be 1.0359 and gallic acid in *Emblica officinalis* Gaertn. of Trichy zone was found to be 0.8016 %. It is concluded that *Emblica officinalis* Gaertn. of Thanjavur zone contain more gallic acid.

UV visible showed (Fig 1,2) absorbance maximum at 423 nm, 382 nm (Thanjavur), 403 nm, 399 nm, 395, 352 nm and 295 nm (Trichy) suggesting the presence of flavonoid class of compounds.

GCMS study on *Emblica officinalis* Gaertn. revealed totally different compounds from the fractions of Trichy and Thanjavur samples. At the same time the presence of some common compounds in both the samples also given in the table 7.

A conclusion was arrived on the basis of chemical standardization of fruits of *Emblica officinalis* Gaertn. collected from Thanjavur and

Trichy that the Trichy sample proved to be a therapeutics better due to the rich content of flavones, alkaloids and Tannins. From the purity point of view, Thanjavur sample was found to be better. It was concluded that environmental contamination and storage

played considerable role in deciding the chemical potentials and efficacy. Standardization and validation studies followed in evaluating standard& purity of the raw materials resulting in identification of the quality of herbal products.

**Table 1: Extractive Value of *Emblica officinalis* Gaertn. in Thanjavur, Trichy Sample**

S. No.	Extract	Concentration (%) (Thanjavur)	Concentration (%) (Trichy)
1	Chloroform	0.737	0.042
2	Hexane	0.15	0.094
3	Alcohol	9.2	0.061

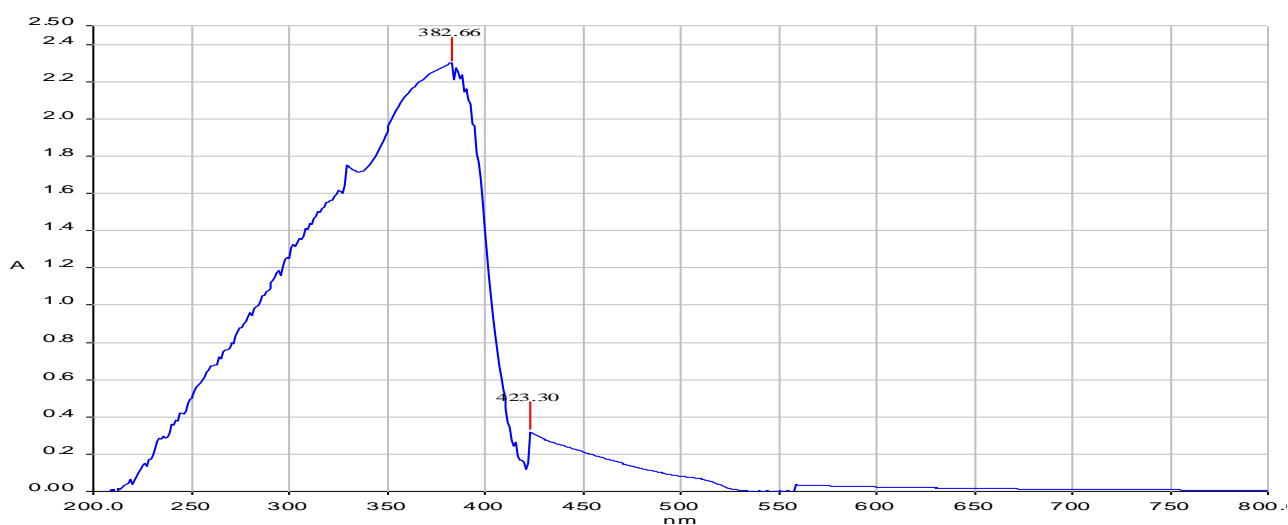
**Table 2: Test for Purity. Physicochemical data of *Emblica officinalis* Gaertn. in Thanjavur, Trichy Sample**

S. No.	Physico chemical constants	Concentration (%) (Thanjavur)	Concentration (%) (Trichy)
1	Loss on Drying	12.75	5.24
2	Ash value	5.32	3.26
3	Acid insoluble	0.71	0.32
4	Water soluble	2	1.5
5	Sulphated ash	6	4

**Table 3: Preliminary phytochemical Screening of *Emblica officinalis* Gaertn. In Thanjavur sample**

S. No.	Compounds	Chloroform	Hexane	Alcohol
1	Phenolic compounds	-	-	+
2	Reducing Sugar	+	+	+
3	Carbohydrates	-	-	+
4	Flavones	+	-	+
5	Glycosides	+	+	-
6	Saponins	+	+	+
7	Steroids	-	-	-
8	Alkaloids	+	+	+
9	Quinones	+	-	+
10	Amino acids	-	-	-
11	Proteins	-	-	-
12	Tannins	+	+	+

+ : Presence ; - : Absence



Spectrum Name: C:\UVWINLAB\DATA\EO.SP

Scan Speed: 240.00 nm/n

Date Created: Thu Feb 16 14:32:37 2012

Slit Width: 1.0000 nm

Instrument Model: Lambda 25

Smooth Bandwidth: 0.00

**Fig. 1: UV-visible spectrum: Sample name: *Emblica officinalis* Gaertn. ( Thanjavur)**

Table 4: Fluorescence Analysis of *Emblica officinalis* Gaertn. in Thanjavur Sample

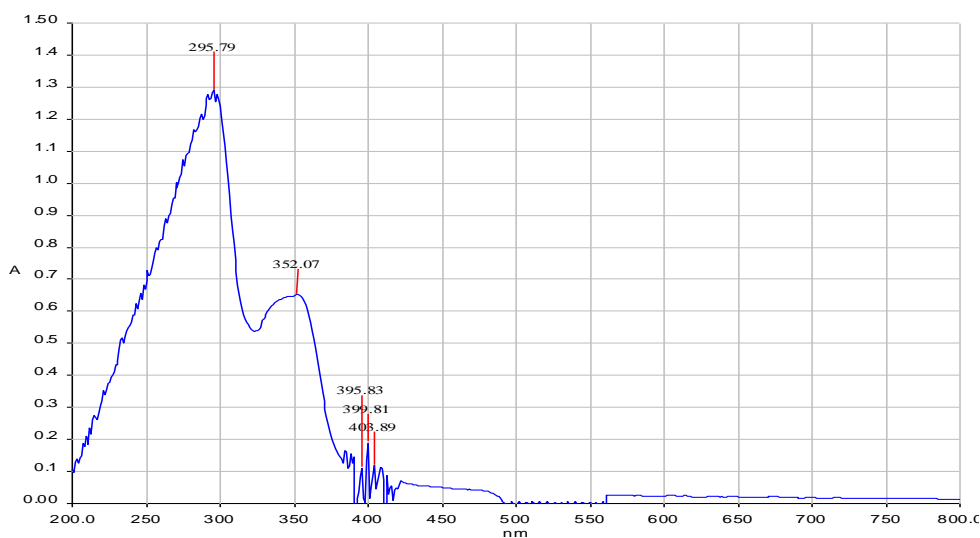
S. No.	Materials	Day light	254nm(UV visible)	366nm(UV visible)
1	Powder	Light brown	Brown	Brown
2	Powder + H <sub>2</sub> SO <sub>4</sub>	Dark brown	Dark brown	Light brown
3	Powder + Nitric acid	Yellowish Orange	Yellowish Orange	Pale yellow
4	Powder + HCL	Brown	Dark brown	Brown
5	Powder + NaOH	Brown	Light brown	Light brown
6	Powder + CH <sub>3</sub> COOH	Brown	Brown	Brown
7	Powder + Iodine	Yellowish brown	Brown	Brown
8	Powder + FeCl <sub>3</sub>	Dark blue	Black	Black
9	Powder + Picric acid	Light Brown	Yellow brown	Brown
10	Powder + NH <sub>4</sub> OH	Brown	Brown	Brown

Table 5: Quantitative Phytochemical analysis of *Emblica officinalis* Gaertn. in Thanjavur, Trichy Sample

S. No.	Estimation	Percentage (%) (Thanjavur)	Percentage (%) (Trichy)
1	Alkaloid	0.040	0.054
2	Flavonoid	11.4	14.5
3	Tannin	13.77	18.54

Table 6: X-ray Fluorescence Spectrometer (XRF) analysis of *Emblica officinalis* Gaertn. - Thanjavur, Trichy.

Formula	Concentration (%) (Thanjavur)	Concentration (%) (Trichy)
K	65.58	60.62
Cl	8.37	8.13
Ca	11.86	12.71
P	2.09	2.29
S	1.86	1.66
Mo	1.39	1.25
Mg	0.69	0.83
Si	2.09	4.58
Fe	2.33	3.54
Al	0.69	1.45
Zn	0.23	-
Pd	1.39	1.45
Na	1697 PPM	-
Cu	1744 PPM	-
Ru	-	0.63
Ti	-	0.42
Mn	-	0.42



Spectrum Name: C:\UVWINLAB\DATA\EOTRI.SP

Scan Speed: 240.00 nm/r

Date Created: Fri Feb 17 15:14:31 2012

Slit Width: 1.0000 nm

Instrument Model: Lambda 25

Smooth Bandwidth: 0.00 nm

Fig. 2: UV-visible spectrum: Sample name: *Emblica officinalis* Gaertn. (Trichy)

Threshold: 0.1000

S. No.	Abscissa nm	Ordinate A
1	423.30	0.3291
2	382.66	2.3060

Threshold: 0.1000

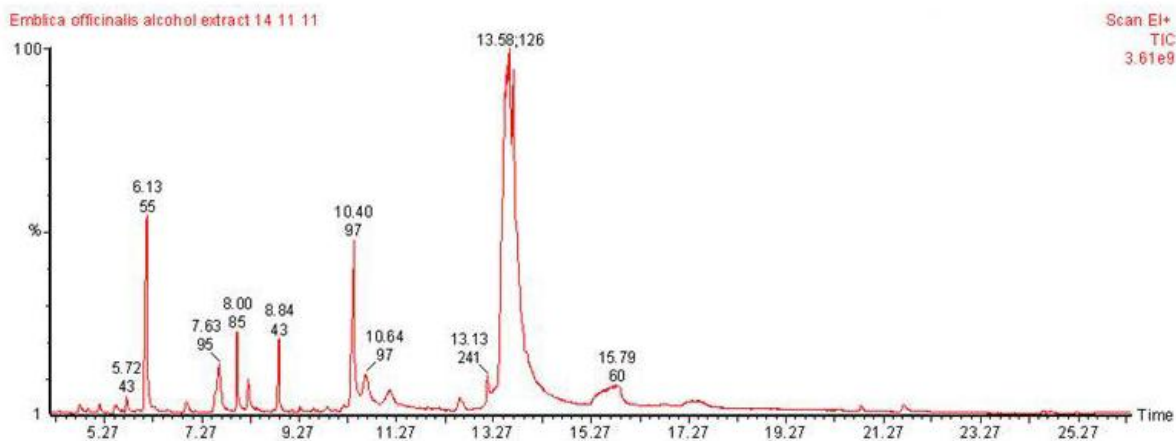
S. No.	Abscissa nm	Ordinate A
1	403.89	0.1193
2	399.81	0.1941
3	395.83	0.1122
4	352.07	0.6520
5	295.79	1.2911

Table 7: Gas Chromatography Linked Mass Spectrophotometer (GCMS) *Emblica officinalis* Gaertn. alcohol extract - Thanjavur

Peak Name	Retention time	Peak area	%Peak area
Name: 2-Amino-oxazole Formula: C3H4N2O MW: 84	4.75	3879351	0.2245
Name: 1,2-Cyclopentanedione Formula: C5H6O2 MW: 98	4.93	1553010	0.0899
Name: 2,5-Furandione, dihydro-3-methylene- Formula: C5H4O3 MW: 112	5.17	3607132	0.2087
Name: Methyl 2-furoate Formula: C6H6O3 MW: 126	5.64	426991	0.0247
Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: C6H8O4 MW: 144	5.72	6386979	0.3695
Name: Phenol Formula: C6H6O MW: 94	5.94	1017556	0.0589
Name: 2(5H)-Furanone Formula: C4H4O2 MW: 84	6.13	105114232	6.0818
Name: 1H-Pyrrole, 2,5-dihydro- Formula: C4H7N MW: 69	6.57	1915953	0.1109
Name: 4-Methoxycarbonyl-4-butanolide Formula: C6H8O4 MW: 144	8.00	32311438	1.8695
Name: 4-Heptanone, 2,6-dimethyl- Formula: C9H18O MW: 142	8.39	1288369	0.0745
Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4 MW: 144	8.84	31224788	1.8066
Name: Benzenecarboxylic acid Formula: C7H6O2 MW: 122	9.28	3571438	0.2066
Name: 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: C6H6O4 MW: 142	9.56	1525841	0.0883
Name: Pyrrolin-2-one-5-methanol, N-methyl- Formula: C6H11NO2 MW: 129	10.00	921567	0.0533
Name: Propanoic acid, 2-methyl-, pentyl ester Formula: C9H18O2 MW: 158	10.19	5372068	0.3108
Name: Malic Acid Formula: C4H6O5 MW: 134	11.12	64700960	3.7435
Name: Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: C9H10O2 MW: 150	11.74	3340164	0.1933
Name: Benzaldehyde, 2-hydroxy-4-methoxy- Formula: C8H8O3 MW: 152	12.13	4451325	0.2575

Name: 1,2,3-Benzenetriol Formula: C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> MW: 126 Pyrogallol	13.58	1321102336	76.4370
Name: D-Allose Formula: C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> MW: 180	15.79	96451064	5.5805
Name: 1,6-Anhydro- $\alpha$ -D-glucofuranose Formula: C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> MW: 162	17.47	32437078	1.8768
Name: Hexadecanoic acid, methyl ester Formula: C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> MW: 270	20.83	2720290	0.1574
Name: 9,12-Octadecadienoic acid, methyl ester Formula: C <sub>19</sub> H <sub>34</sub> O <sub>2</sub> MW: 294	24.56	1075011	0.0622
Name: 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- Formula: C <sub>19</sub> H <sub>32</sub> O <sub>2</sub> MW: 292	24.71	1259281	0.0729
Linolenic acid, methyl ester Name: Octadecanoic acid, methyl ester Formula: C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> MW: 298	25.25	699845	0.0405

**Chromatogram (x-axis = Retention time; y-axis = % intensity/% abundance/ concentration)**

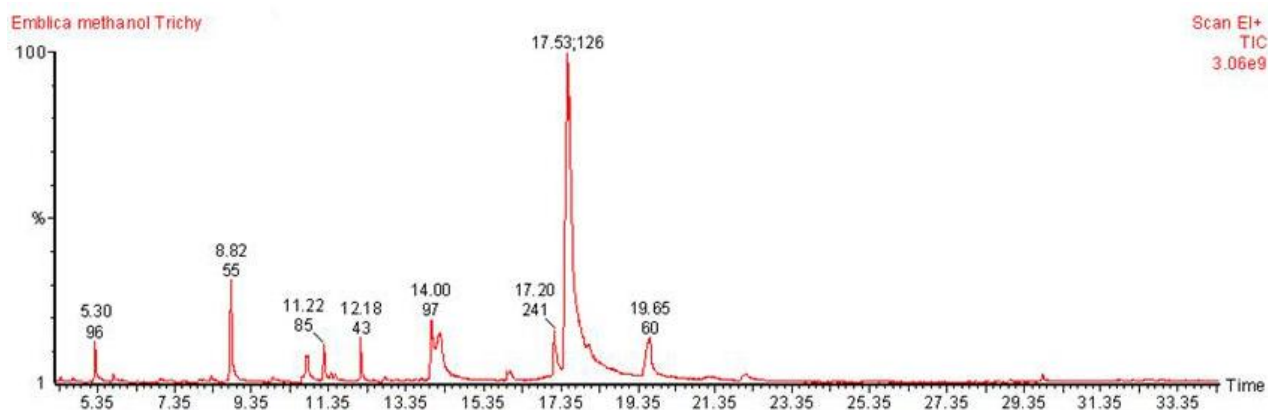


**Gas Chromatography Linked Mass Spectrophotometer (GCMS) analysis of *Emblica officinalis* Gaertn. Methanol extract – Trichy**

Peak Name	Retention time	Peak area	%Peak area
Name: 1H-Imidazole, 4,5-dihydro-2-methyl- Formula: C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> MW: 84 CAS	4.30	410859	0.0425
Name: Butanimidamide Formula: C <sub>4</sub> H <sub>10</sub> N <sub>2</sub> MW: 86	4.41	1821311	0.1885
Name: 2(5H)-Furanone Formula: C <sub>4</sub> H <sub>4</sub> O <sub>2</sub> MW: 84	4.73	2050839	0.2123
Name: Butanoic acid, 3-hydroxy-, methyl ester, (S)- Formula: C <sub>5</sub> H <sub>10</sub> O <sub>3</sub> MW: 118	5.14	343333	0.0355
Name: Furfural Formula: C <sub>5</sub> H <sub>4</sub> O <sub>2</sub> MW: 96	5.30	15075660	1.5604
Name: 5-Amino-3-methylisoxazole Formula: C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> O MW: 98	5.76	6003320	0.6214
Name: Butanoic acid, 3-oxo-, methyl ester Formula: C <sub>5</sub> H <sub>8</sub> O <sub>3</sub> MW: 116	5.99	1761637	0.1823
Name: 2-Amino-oxazole Formula: C <sub>3</sub> H <sub>4</sub> N <sub>2</sub> O	6.99	3846437	0.3981

MW: 84 Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: C5H6O2	7.26	1855555	0.1921
MW: 98 Name: 2,5-Furandione, 3-methyl- Formula: C5H4O3	7.60	1045549	0.1082
MW: 112 CAS Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: C6H8O4	8.29	3732138	0.3863
MW: 144 Name: 1H-1,3-Diazepine, 4,5,6,7-tetrahydro-2-methyl- Formula: C6H12N2	8.82	61587808	6.3748
MW: 112 Name: Pentanoic acid, 4-oxo- Formula: C5H8O3	9.91	2868841	0.2969
MW: 116 Name: 4-Methoxycarbonyl-4-butanolide Formula: C6H8O4	11.22	18057546	1.8691
MW: 144 Name: Levoglucosenone Formula: C6H6O3	11.41	4839146	0.5009
MW: 126 Name: 3-Acetoxy-3-hydroxypropionic acid, methyl ester Formula: C6H10O5	11.50	5051329	0.5229
MW: 162 Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4	12.18	23058636	2.3867
MW: 144 Name: Benzenecarboxylic acid Formula: C7H6O2	12.81	4678855	0.4843
MW: 122 Name: 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: C6H6O4	13.13	1167466	0.1208
MW: 142 Name: 2-Butenoic acid, 4,4-dimethoxy-, methyl ester Formula: C7H12O4	13.28	192081	0.0199
MW: 160 Name: 1,4:3,6-Dianhydro- $\alpha$ -D-glucopyranose Formula: C6H8O4	13.76	877284	0.0908
MW: 144 Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: C6H6O3	14.00	48230068	4.9922
MW: 126 Name: Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: C9H10O2	15.59	363582	0.0376
MW: 150 Name: Phthalic anhydride Formula: C8H4O3	15.80	532292	0.0551
MW: 148 Name: 1,6:3,4-Dianhydro-2-O-acetyl- $\alpha$ -D-galactopyranose Formula: C8H10O5	16.05	6491089	0.6719
MW: 186 Name: 1,2,3-Benzenetriol Formula: C6H6O3	17.53	658047936	68.1129
MW: 126 Name: Pyrogallol Formula: C6H12O6	19.65	62531140	6.4724
MW: 180 Name: $\alpha$ -D-Glucopyranoside, methyl Formula: C7H14O6	21.17	4610500	0.4772
MW: 194 Name: 1,6-Anhydro- $\alpha$ -D-glucofuranose Formula: C6H10O5	22.18	15562962	1.6109
MW: 162 Name: Hexadecanoic acid, methyl ester Formula: C17H34O2	29.01	1529489	0.1583
MW: 270 Name: n-Hexadecanoic acid Formula: C16H32O2	29.83	7472779	0.7735
MW: 256 Name: 9,12-Octadecadienoic acid, methyl ester Formula: C19H34O2	31.76	416258	0.0431
MW: 294			

**Chromatogram** (x-axis = Retention time; y-axis = % intensity/% abundance/ concentration)



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