

Supporting Information

Synthesis and biological activity of human neuropeptide S analogues modified in position 5: identification of potent and pure NPS receptor antagonists

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and Severo Salvadori[§].

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Table 1. analytical properties of the [X⁵]NPS analogues

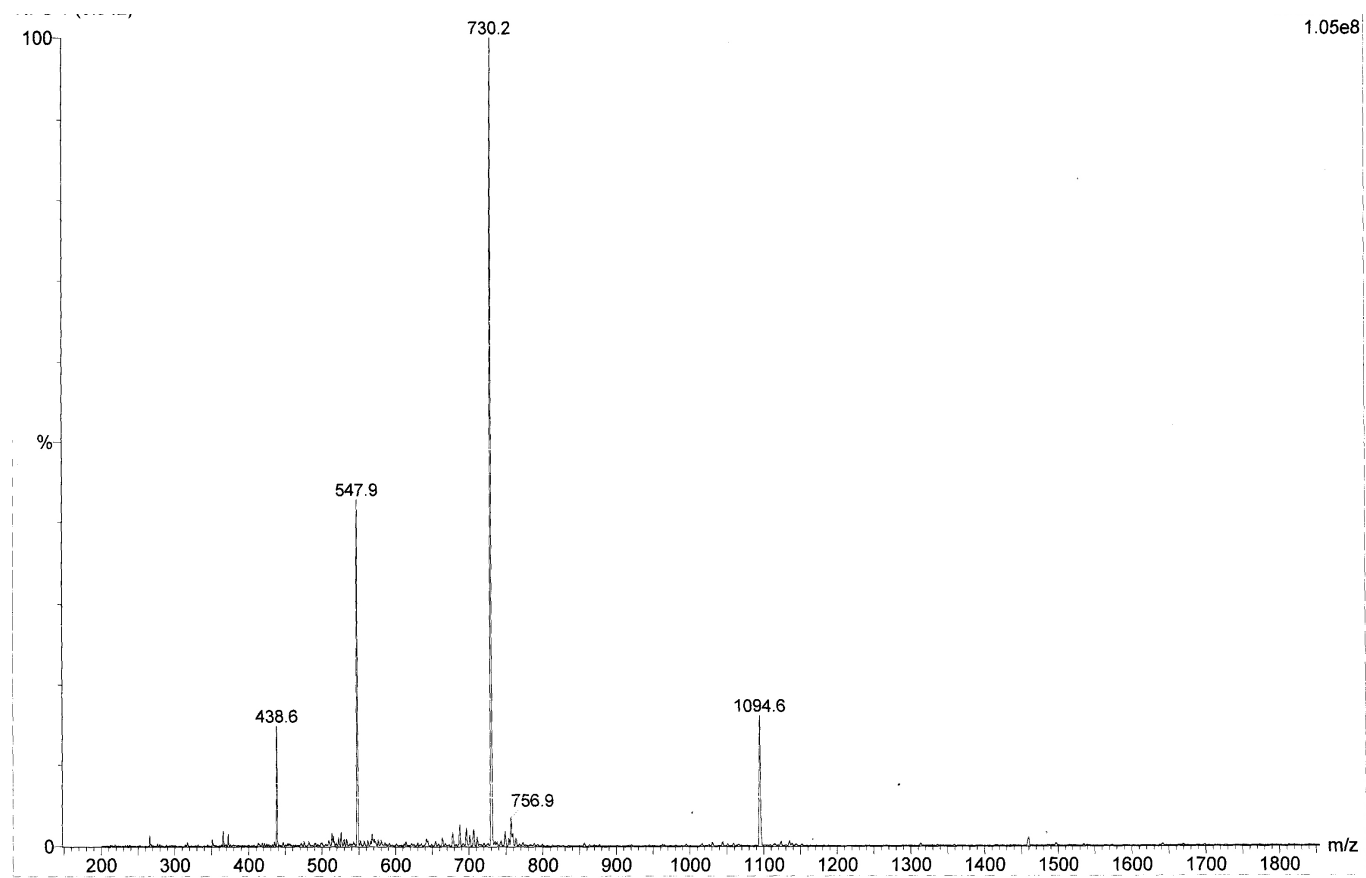
no	Abbreviated names	^a t _r		^b MH ⁺	
		I	II	calculated	found
	hNPS	9.59	13.06	2188.5	2188.2
1	[Phe ⁵]hNPS	10.65	14.14	2278.6	2278.6
2	[Trp ⁵]hNPS	10.96	13.23	2317.7	2317.2
3	[Leu ⁵]hNPS	9.61	13.63	2244.6	2244.8
4	[Val ⁵]hNPS	10.34	14.51	2230.6	2231.0
5	[Met ⁵]hNPS	10.08	13.98	2262.7	2262.2
6	[Cys ⁵]hNPS	10.07	13.92	2235.6	2235.2
7	[D-Phe ⁵]hNPS	10.46	14.22	2278.6	2278.6
8	[D-Trp ⁵]hNPS	10.76	14.30	2317.7	2318.0
9	[D-Leu ⁵]hNPS	9.54	13.69	2244.6	2244.6
10	[D-Val ⁵]hNPS	9.92	13.92	2230.6	2230.8
11	[D-Met ⁵]hNPS	9.75	14.01	2262.7	2263.6
12	[D-Cys ⁵]hNPS	9.98	13.82	2235.6	2235.4
13	[D-Cys(Acm) ⁵]hNPS	10.68	14.08	2307.7	2307.8
14	[D-Cys(Bzl) ⁵]hNPS	10.92	14.52	2324.7	2324.2
15	[D-Cys(tBu) ⁵]hNPS	10.86	14.34	2291.7	2290.4

^at_r is the retention time determined by analytical HPLC. Retention time I was obtained using a Nucleodur C₁₈ column (4.6 x 100 mm, 2 μm particle size) with the solvent system A (10%, v/v, acetonitrile in 0.1% TFA) and solvent system B (60%, v/v, acetonitrile in 0.1% TFA). The column was perfused at a flow rate of 0.6 mL / min using a linear gradient from 0% to 70% B over 25 min.

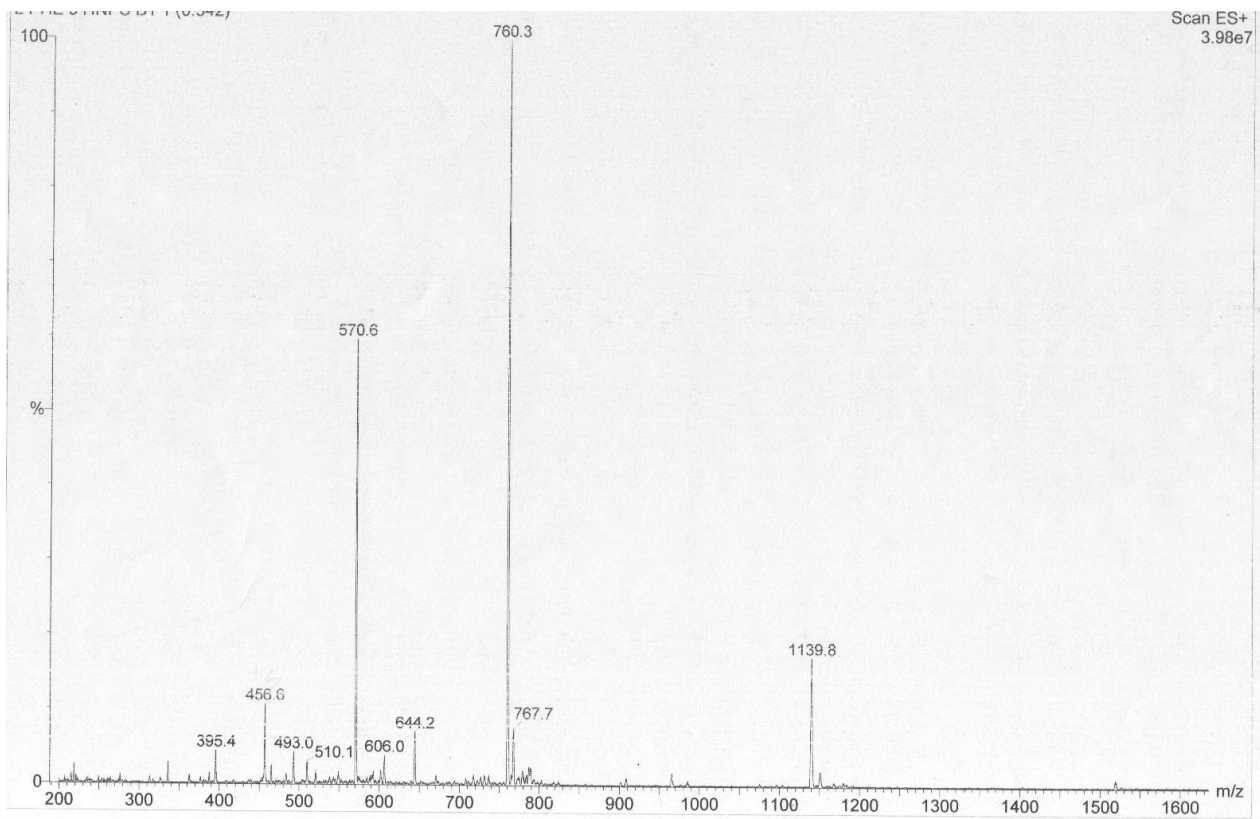
Retention time II was obtained using a Hypersil BDS C₁₈ column (4.6 x 150 mm, 5 μm particle size) with solvent system A (35 mM NaH₂PO₄ (pH 2.1)) and solvent system B (59 mM NaH₂PO₄ (pH 2.1)-acetonitrile (60:40 v/v)). The column was perfused at a flow rate of 1 mL/min with a linear gradient from 5% to 70% B over 25 min

^bThe mass ion (MH⁺) was obtained by electro spray mass spectrometry.

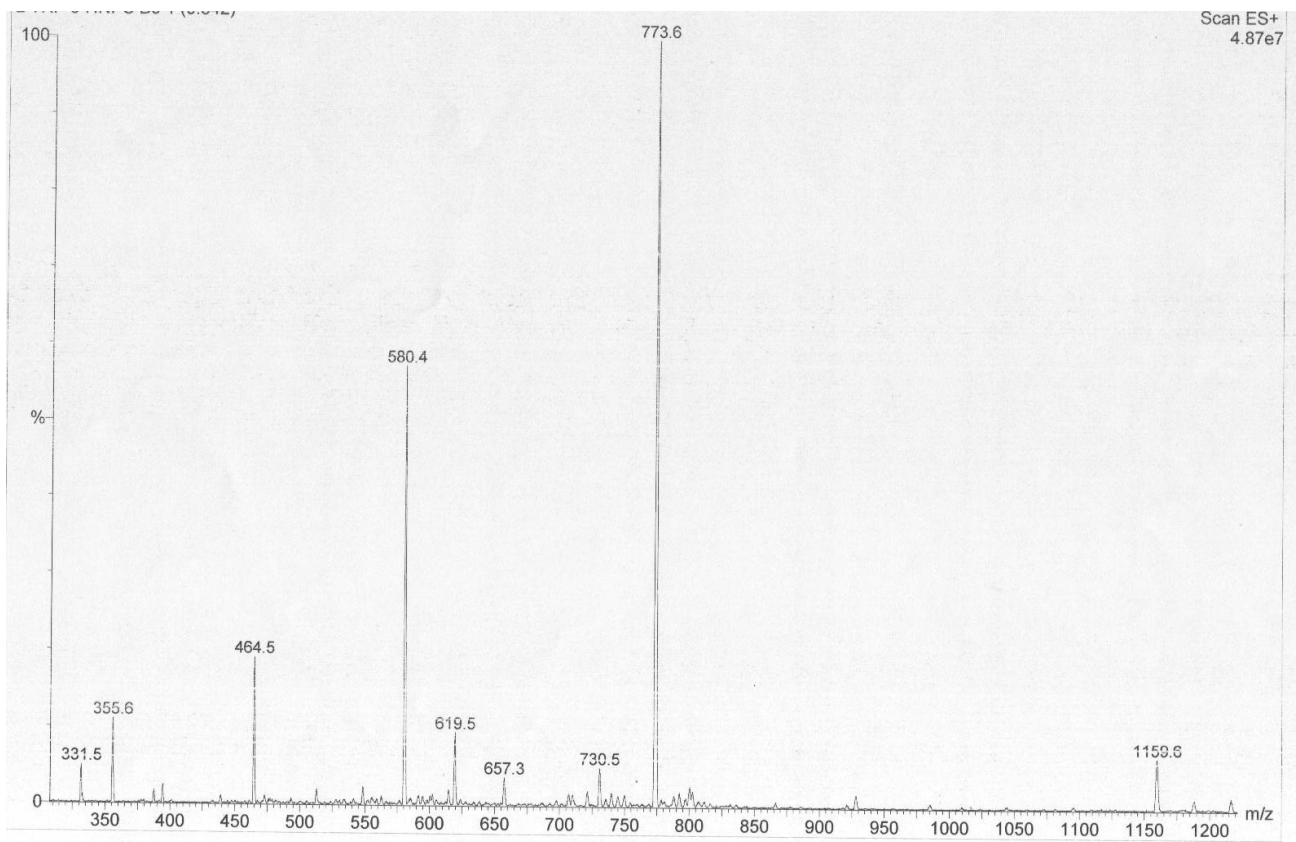
Mass spectra of hNPS analogues



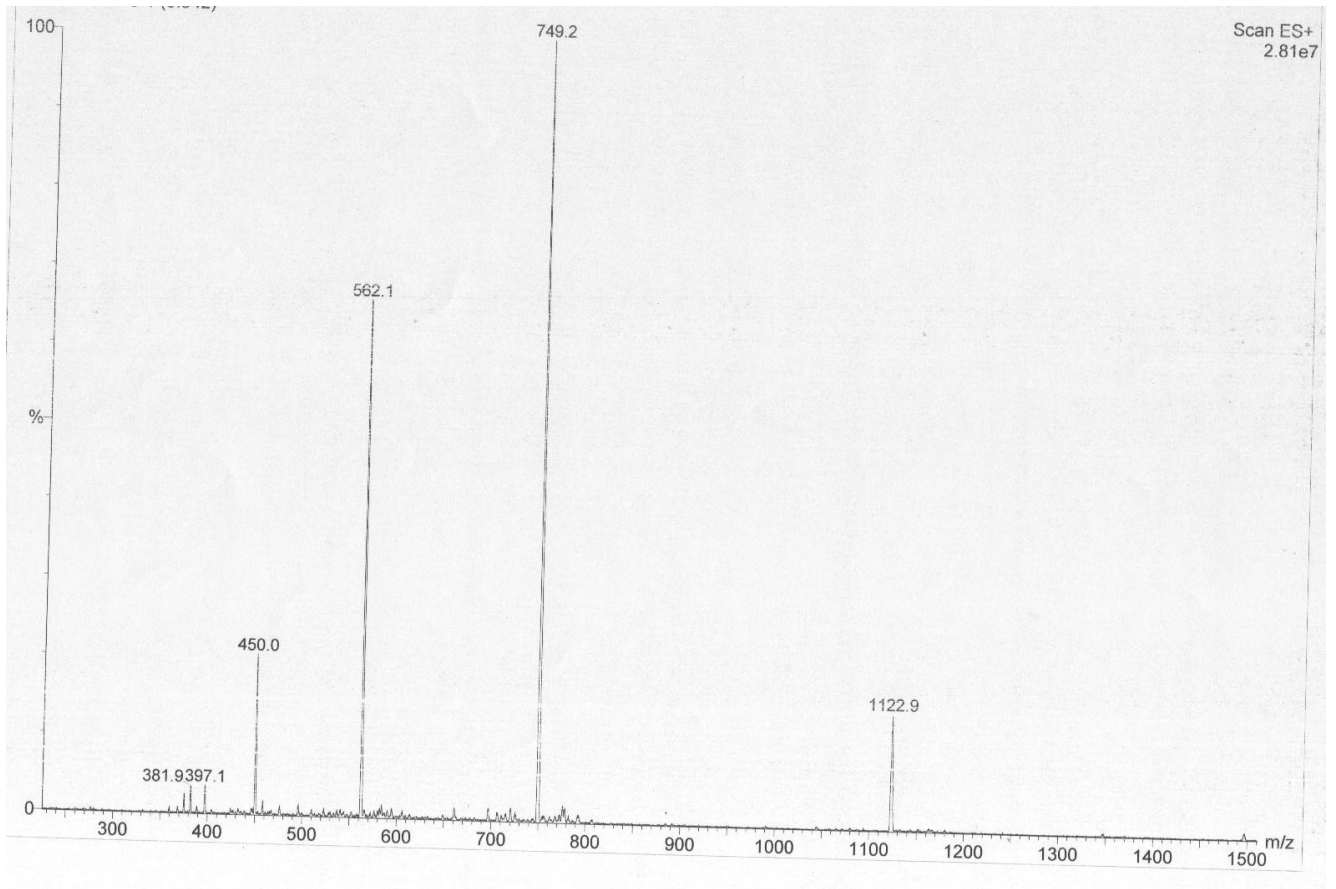
hNPS



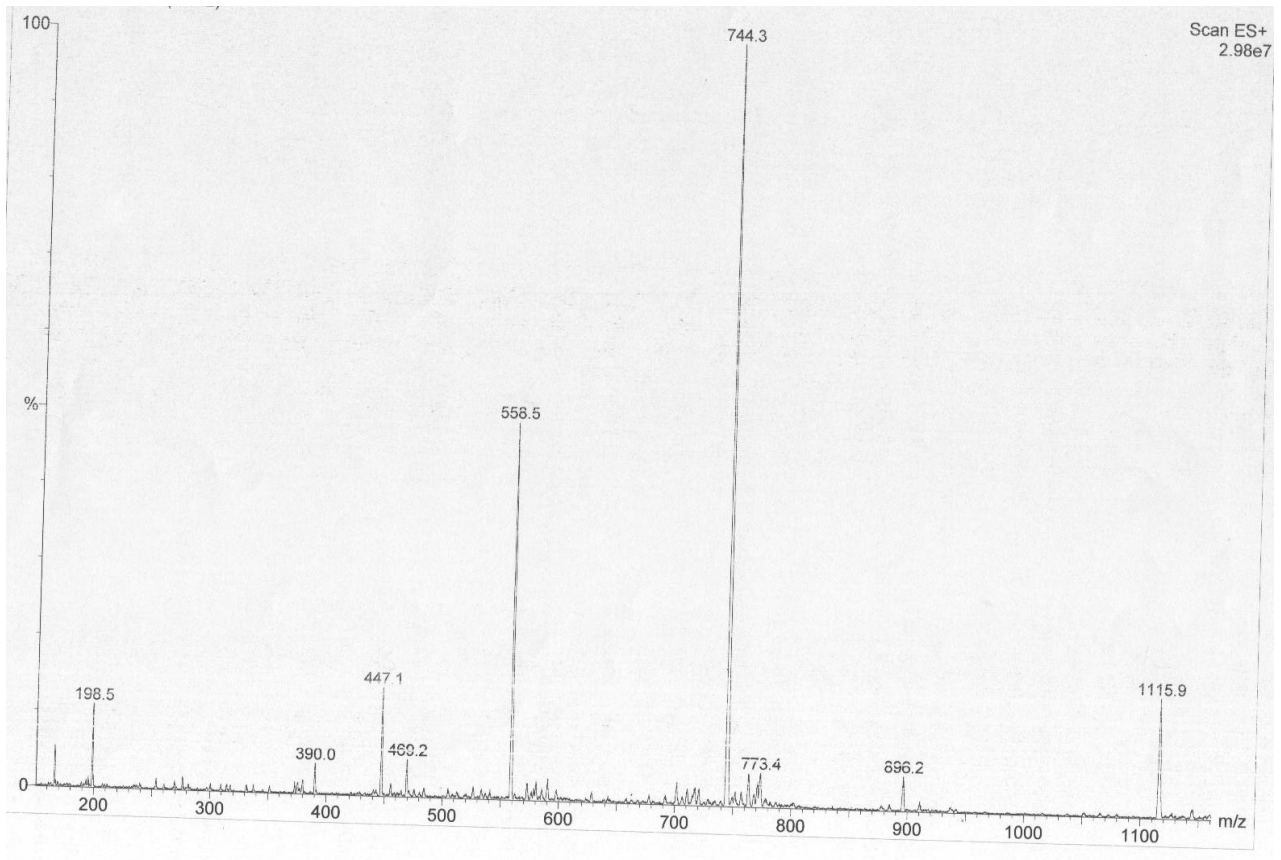
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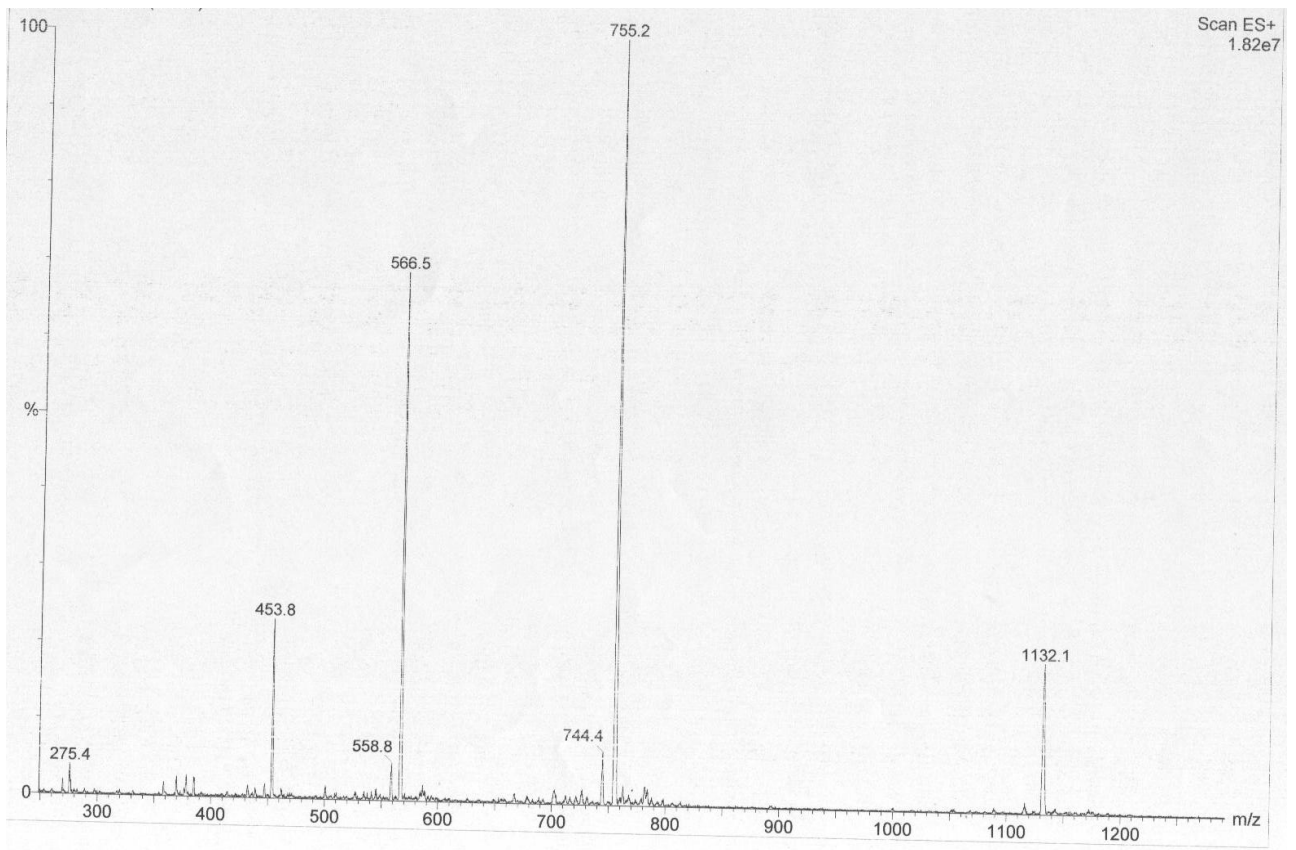
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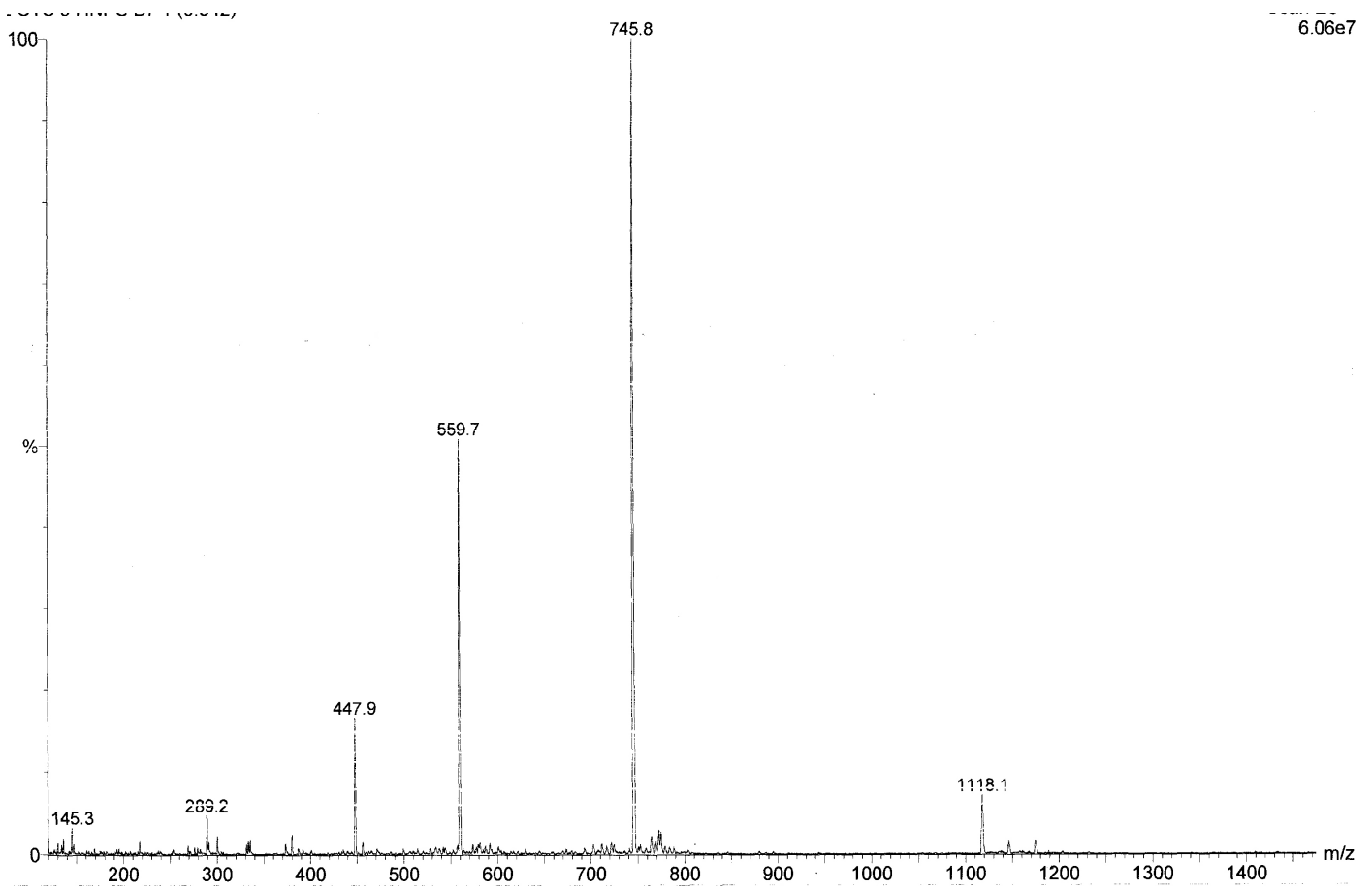
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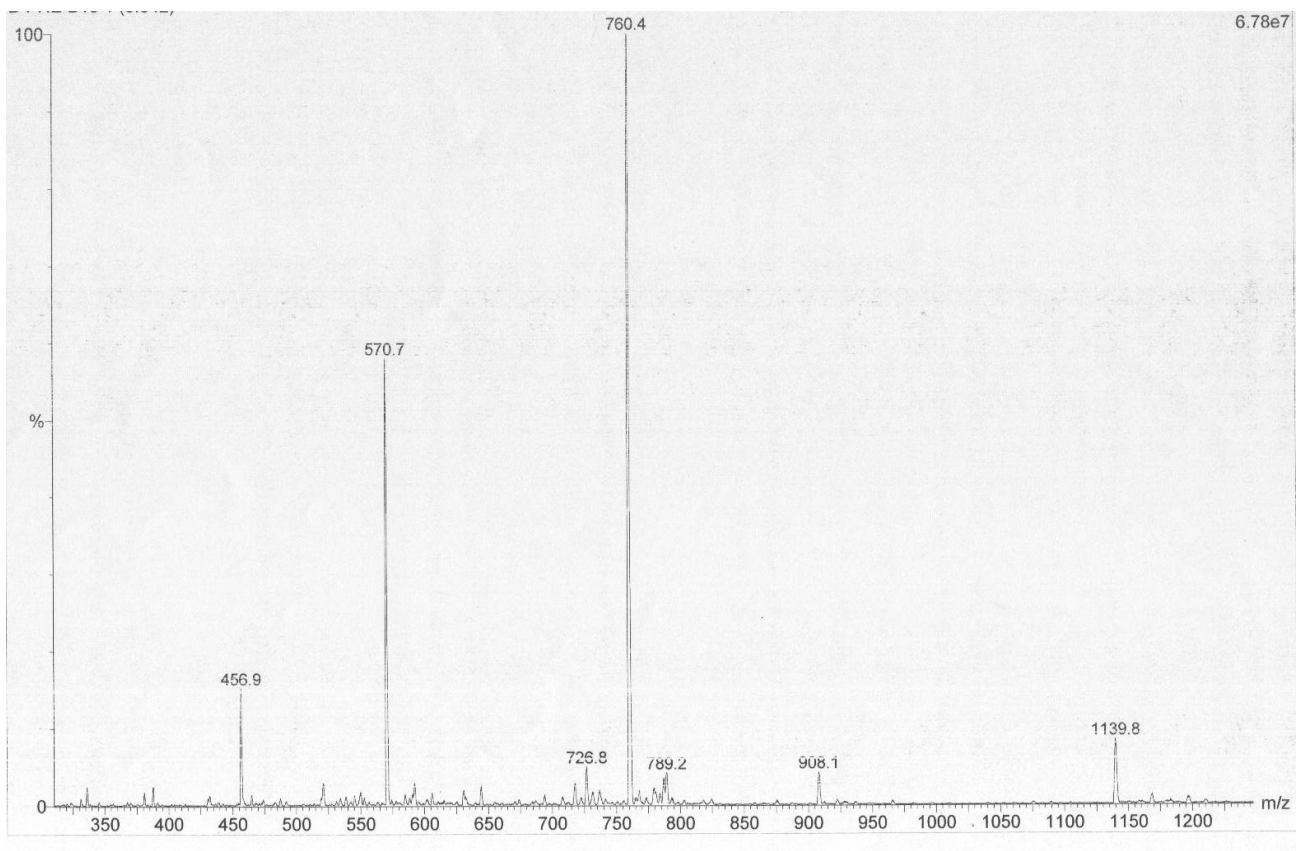
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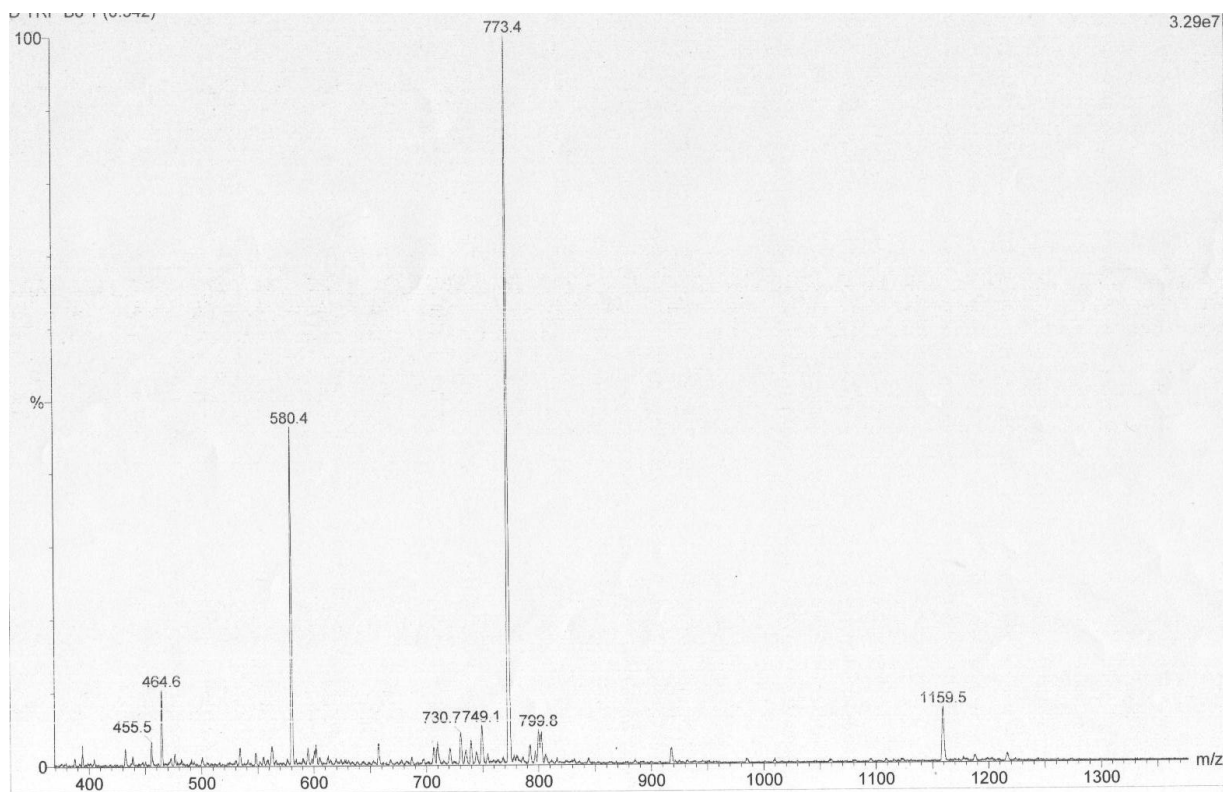
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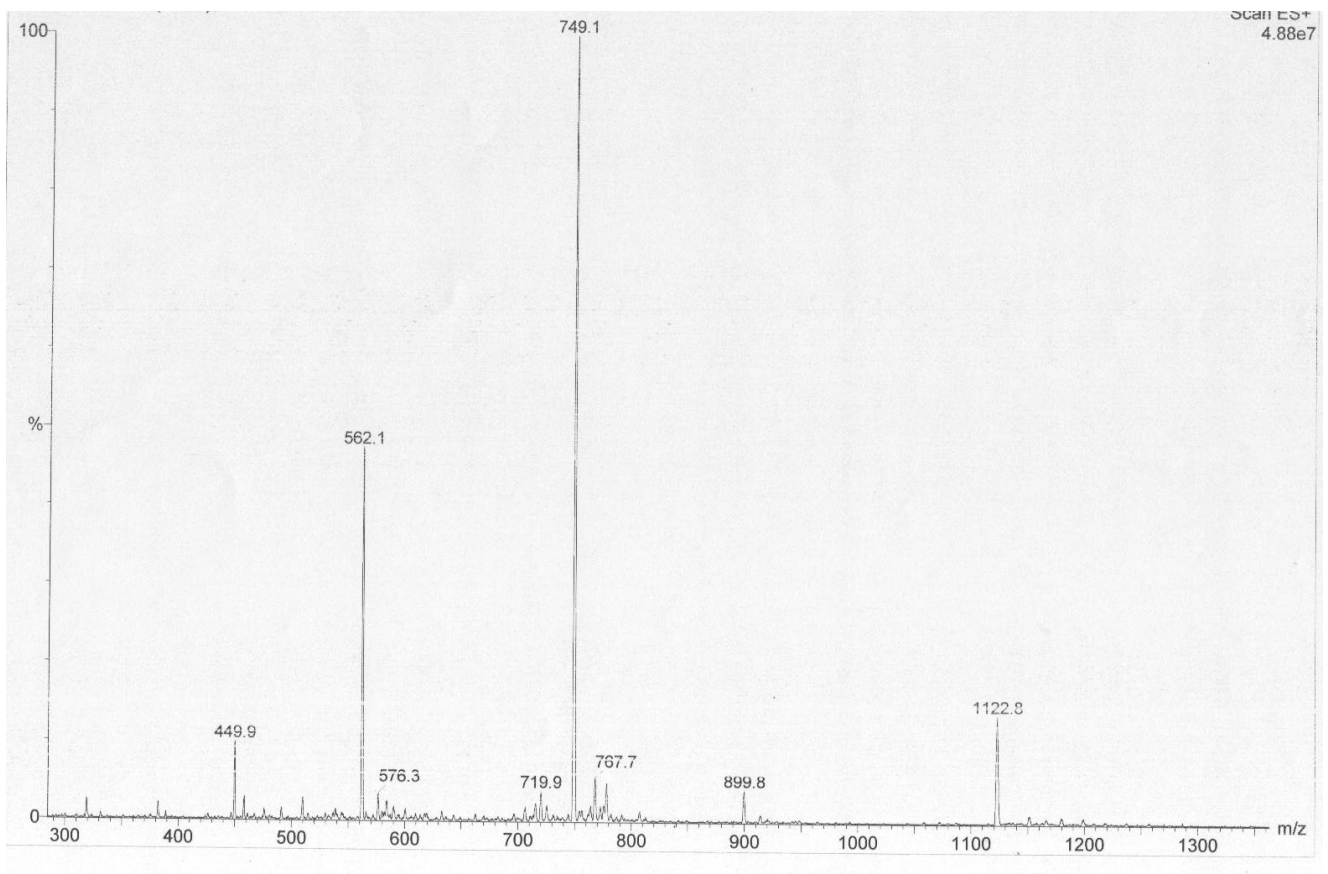
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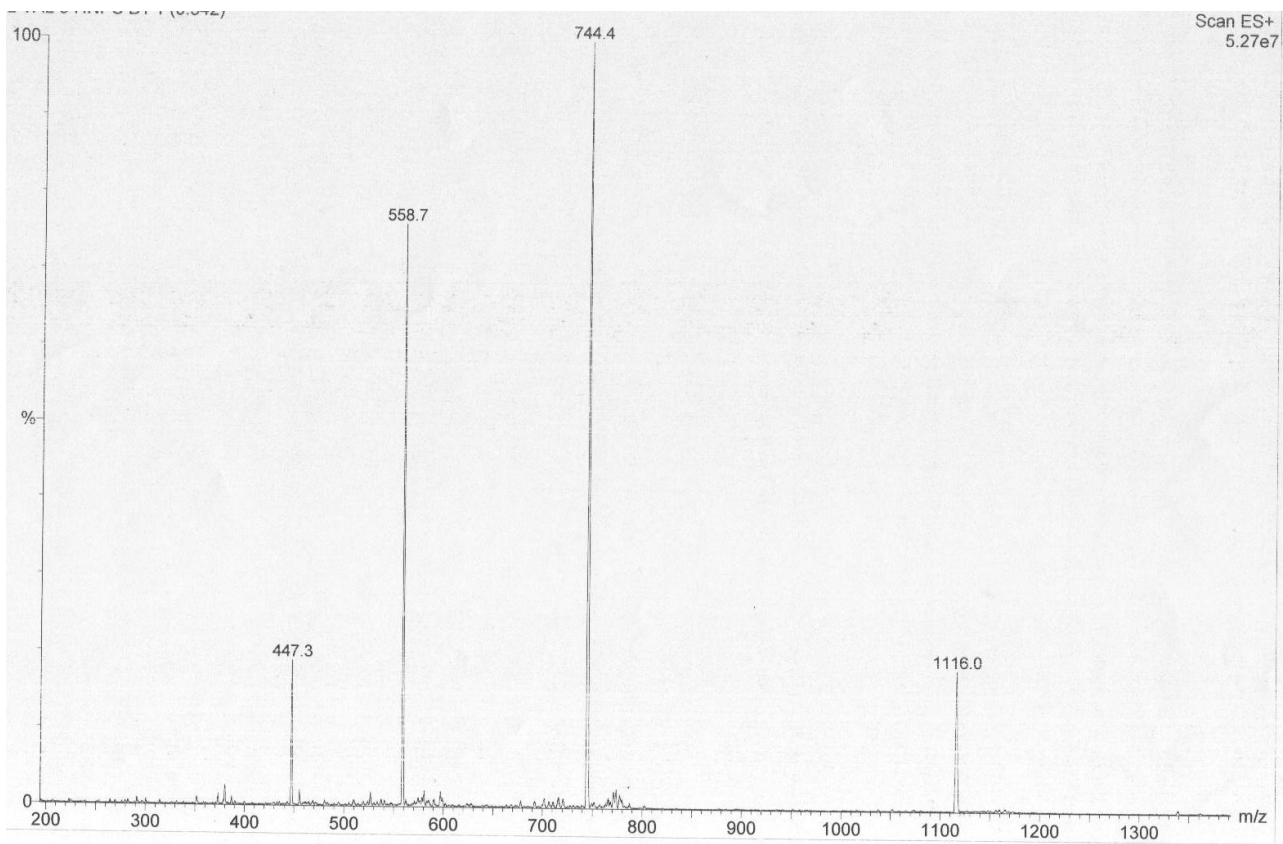
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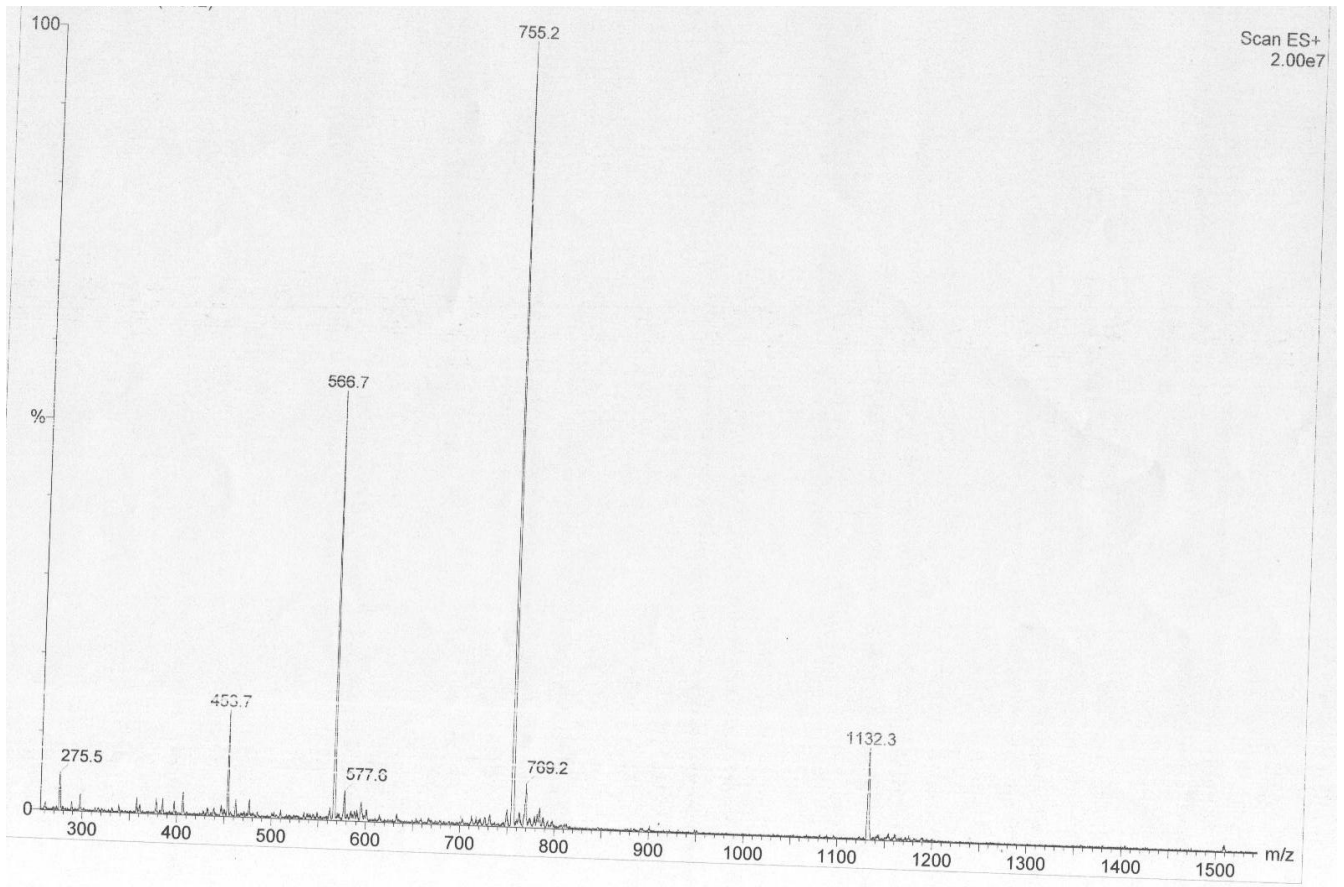
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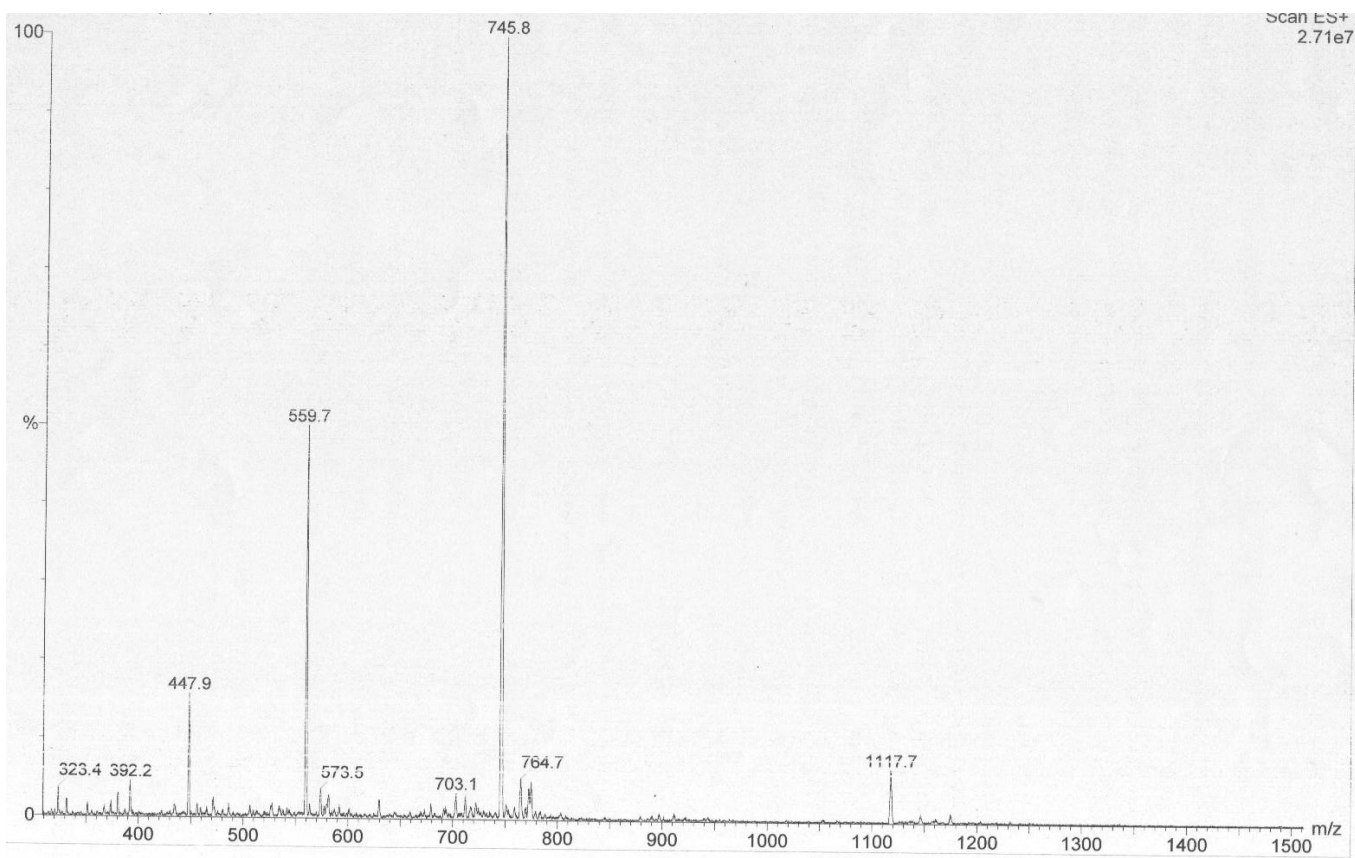
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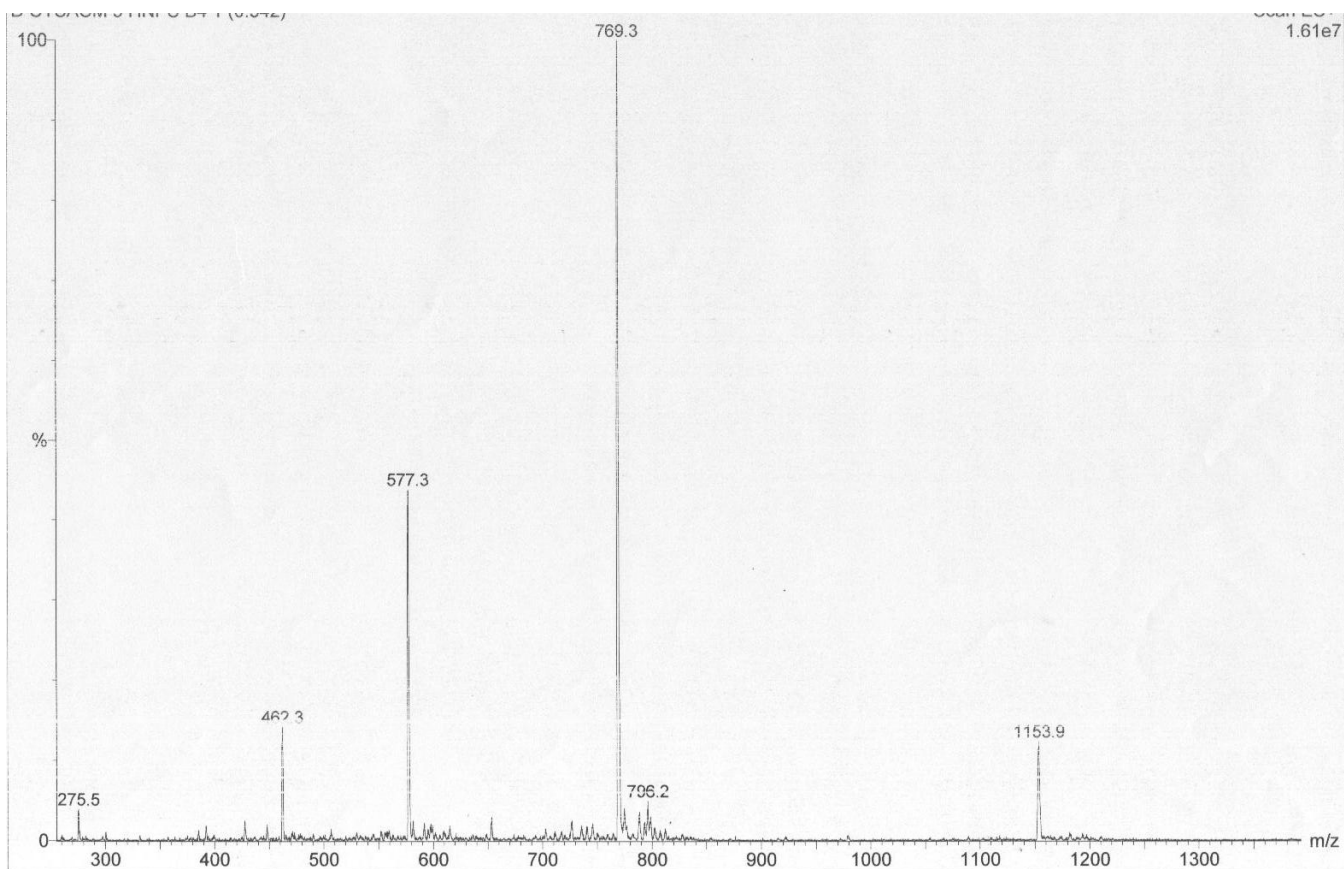
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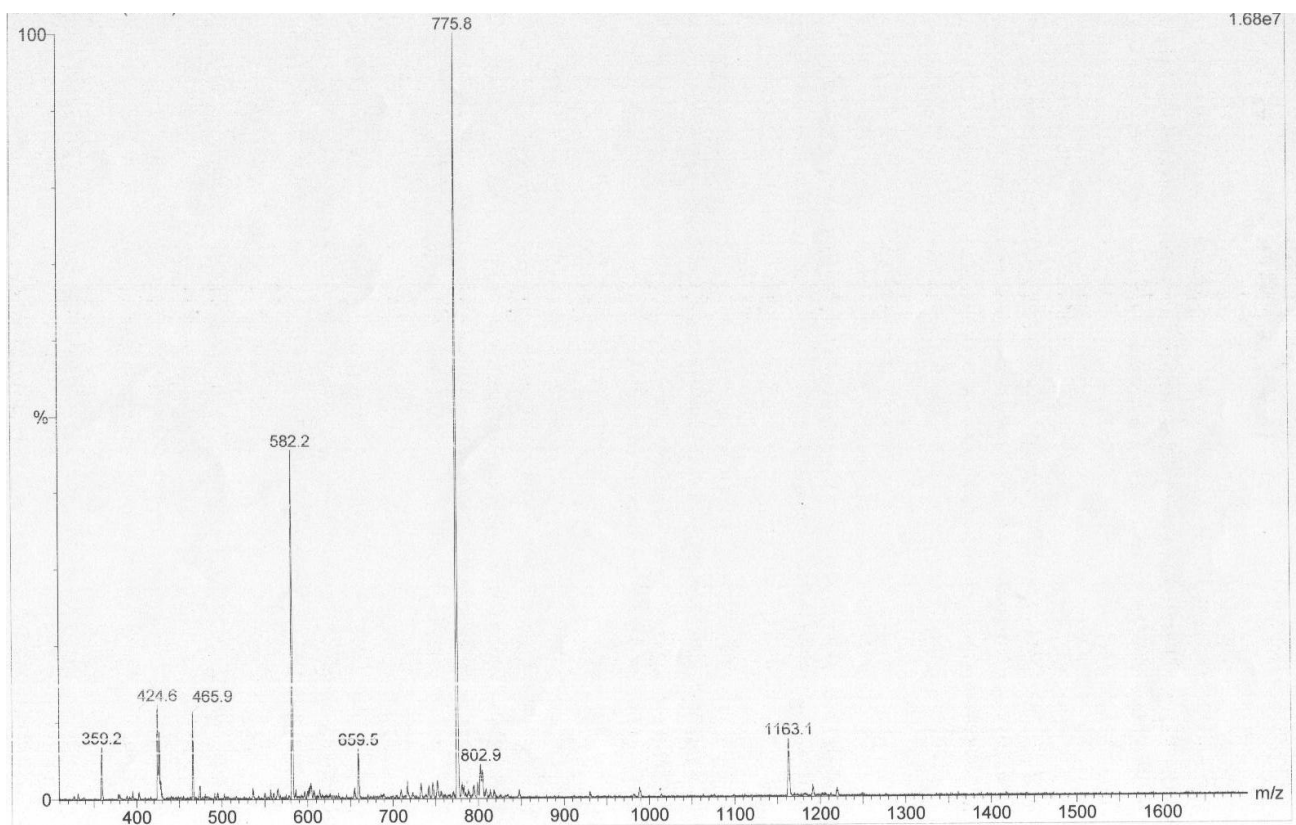
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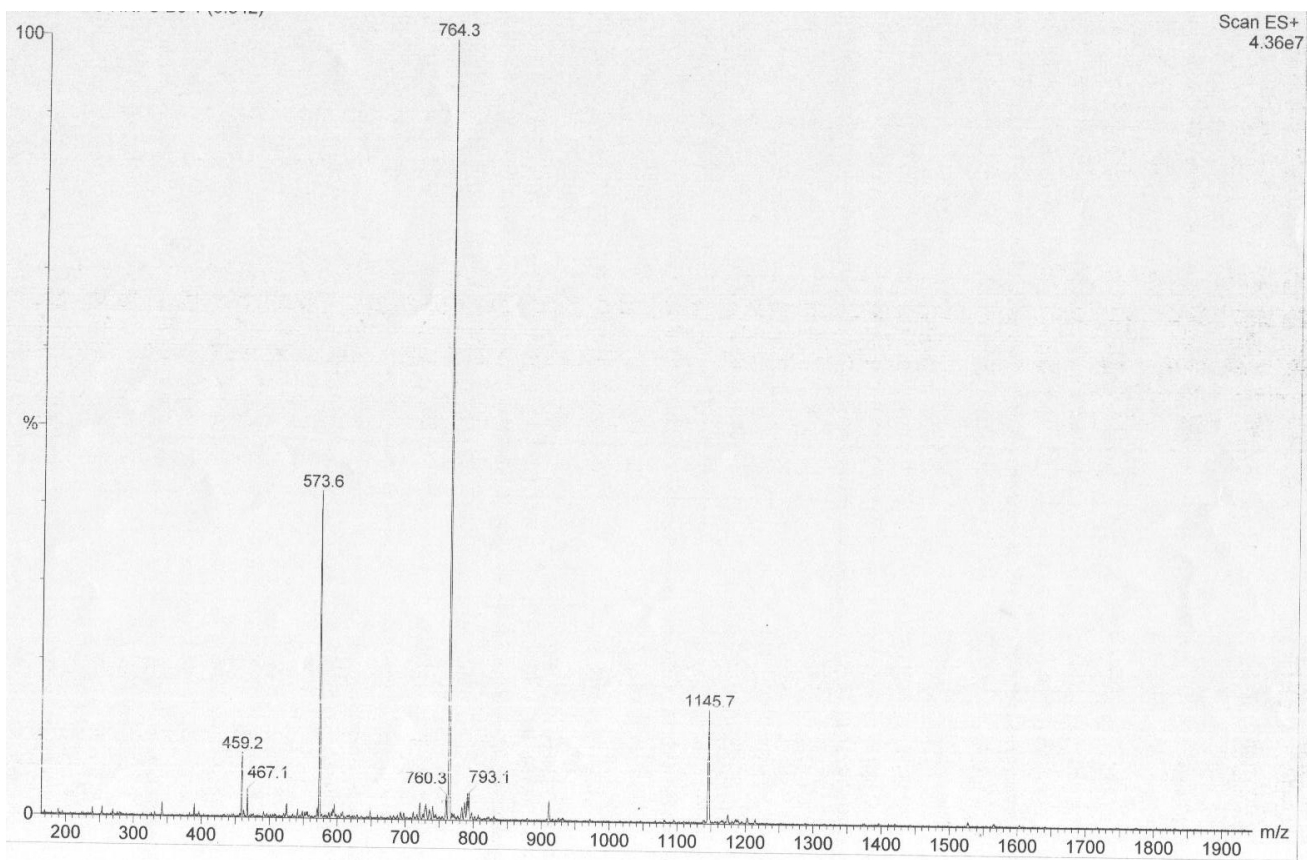
Compound 12



Compound 13

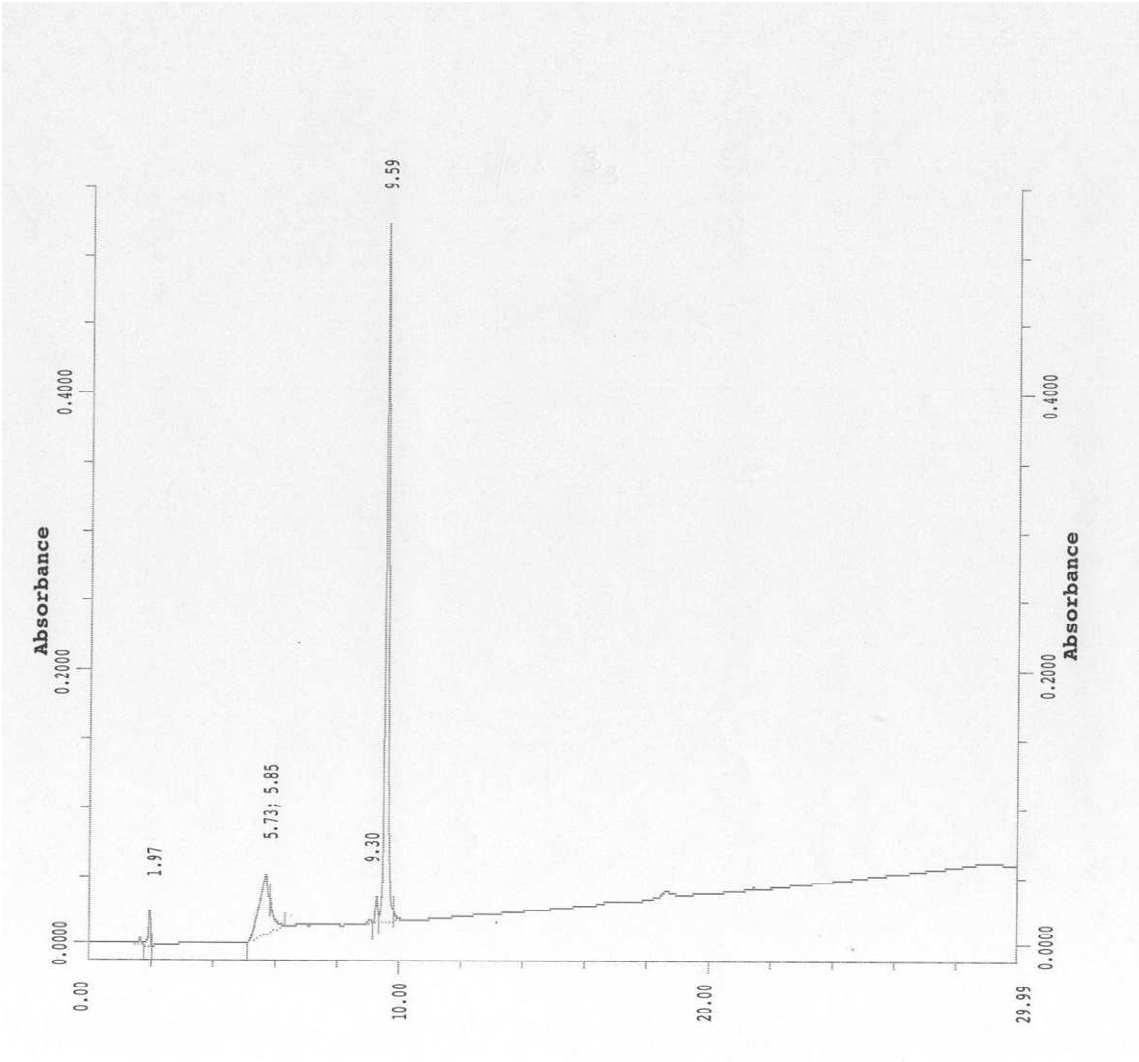


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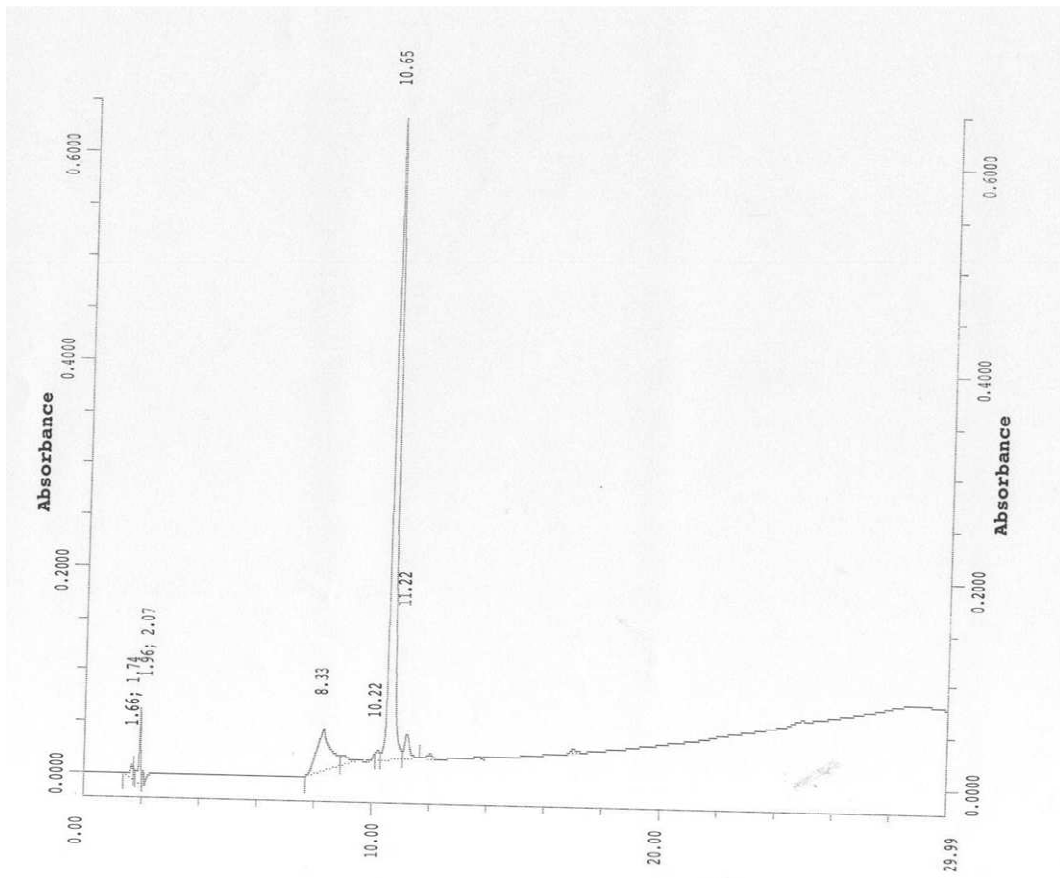


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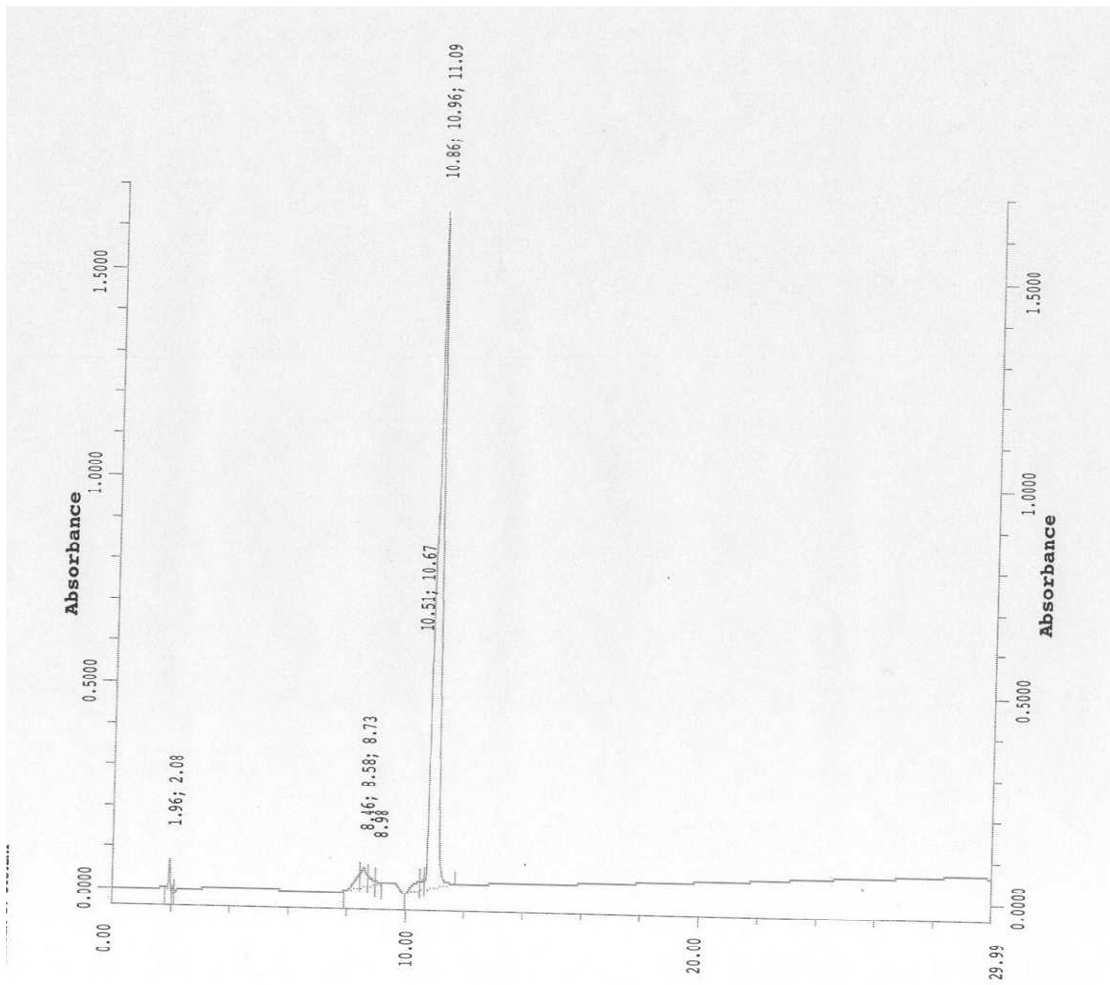
HPLC chromatograms of hNPS analogues (system I)



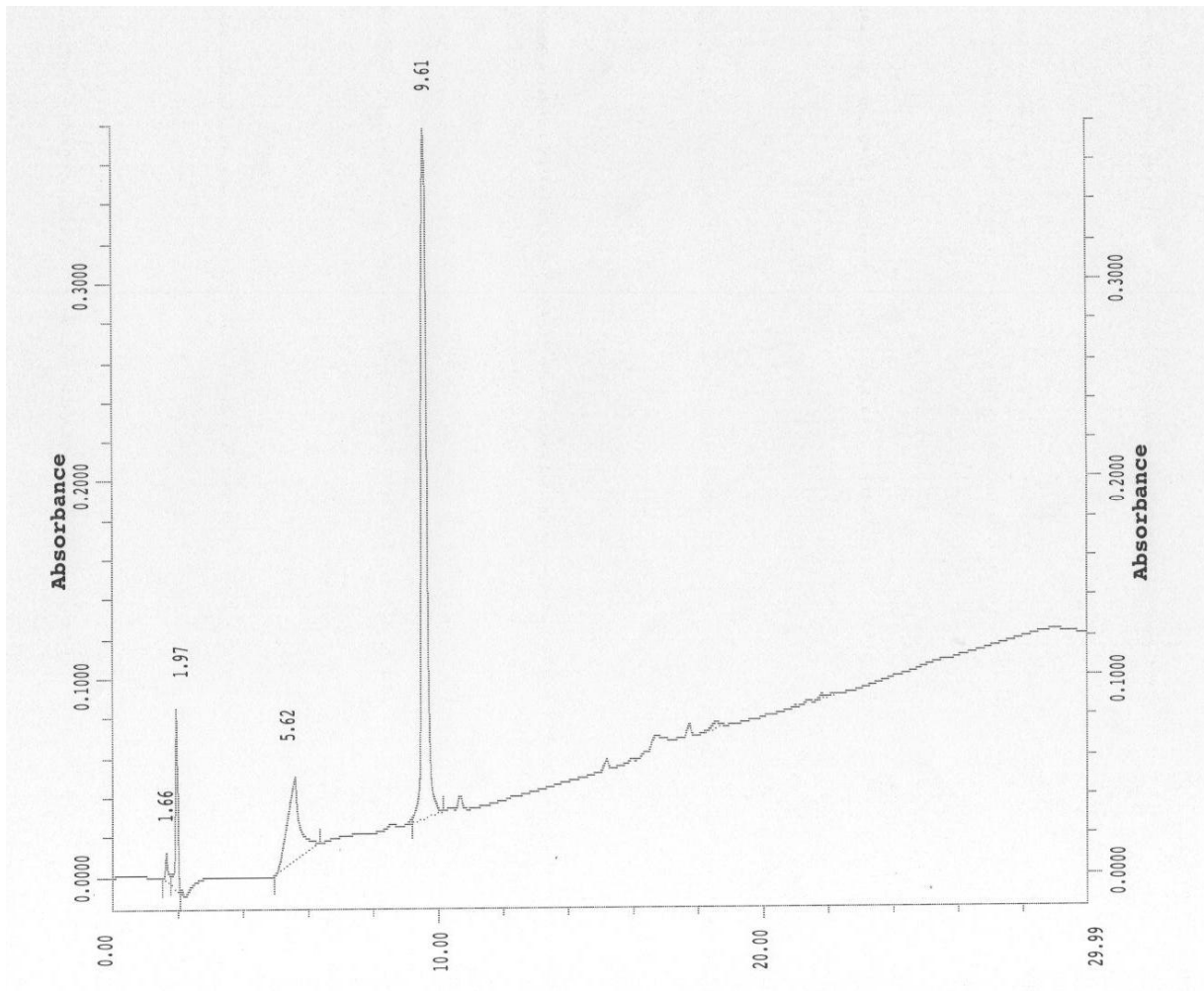
hNPS



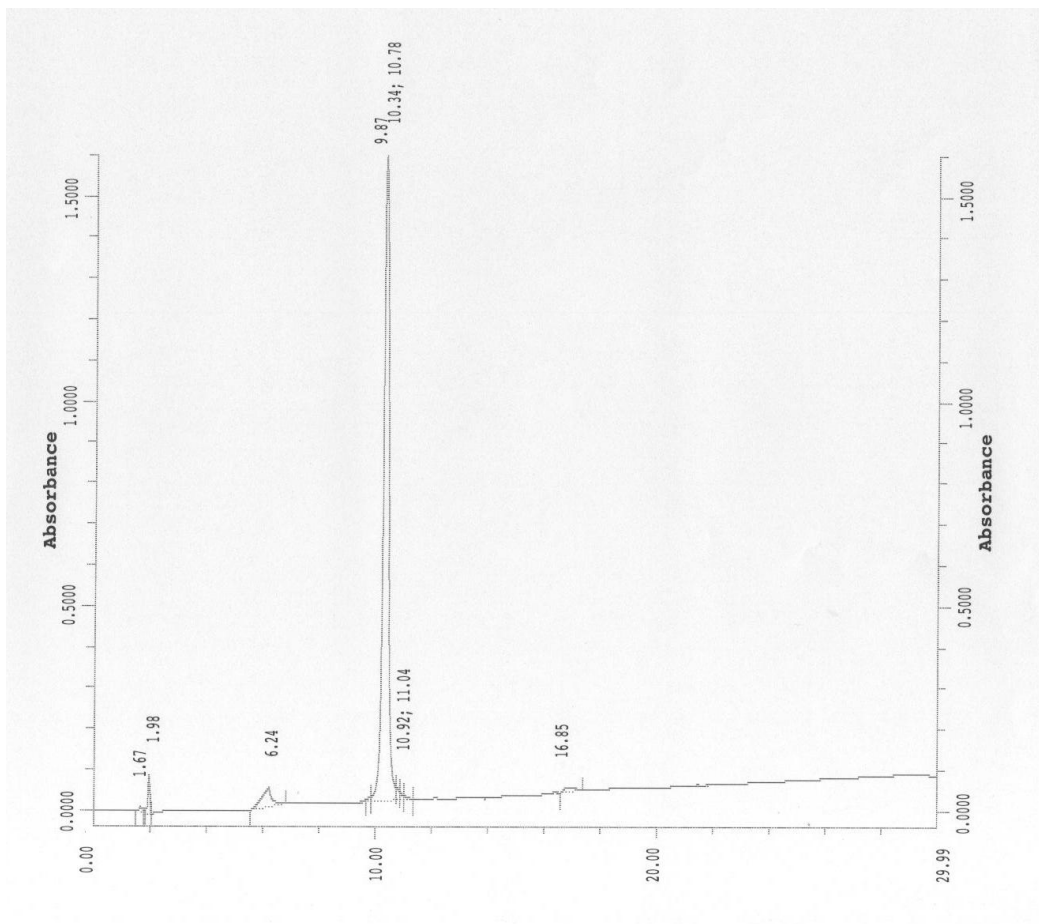
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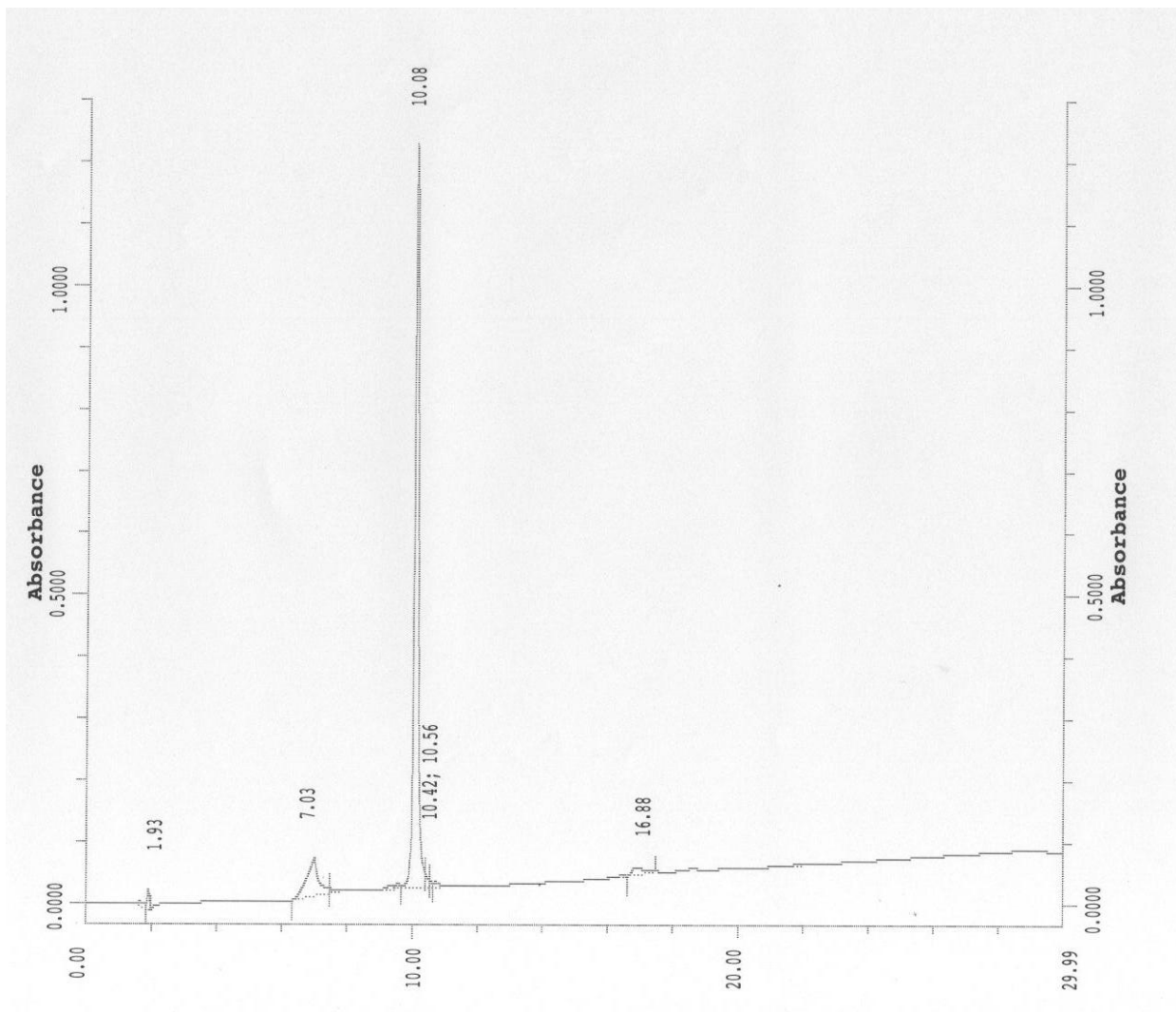
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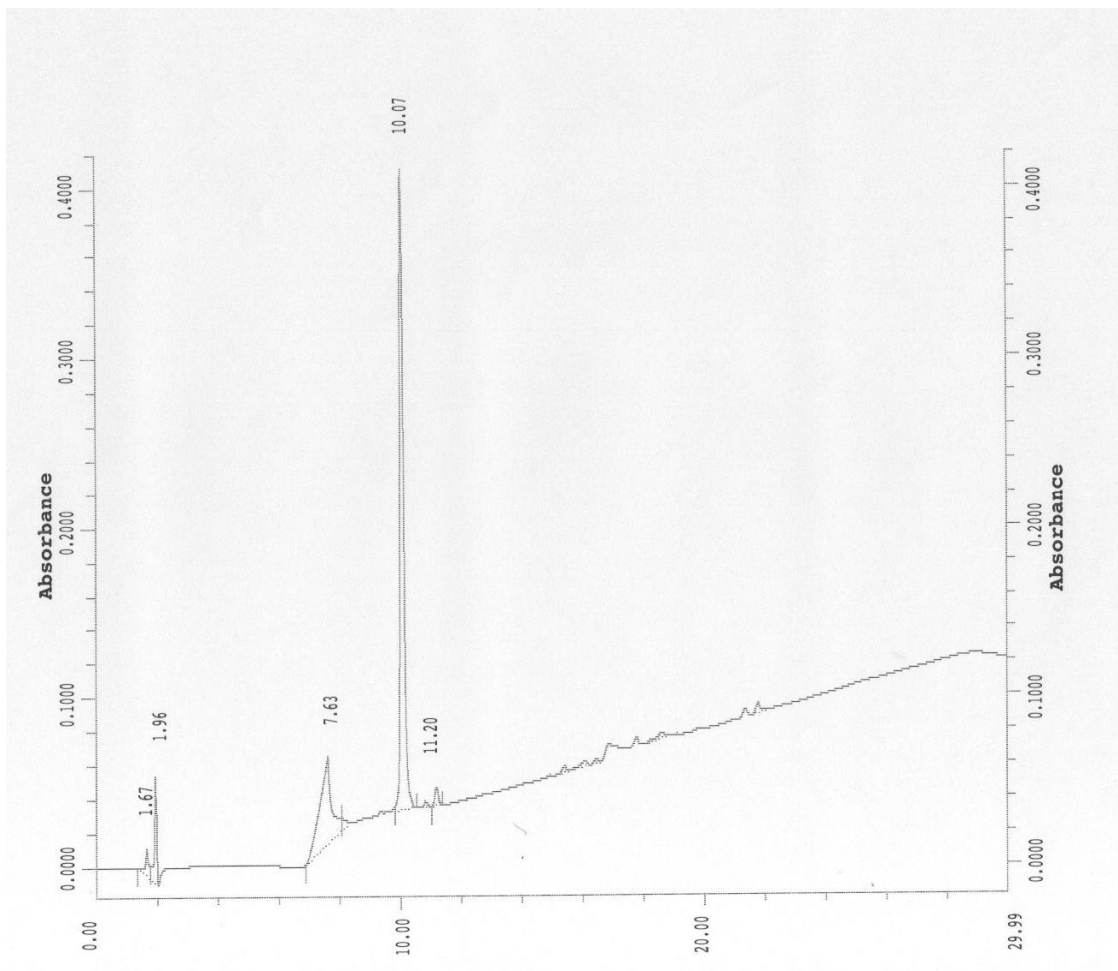
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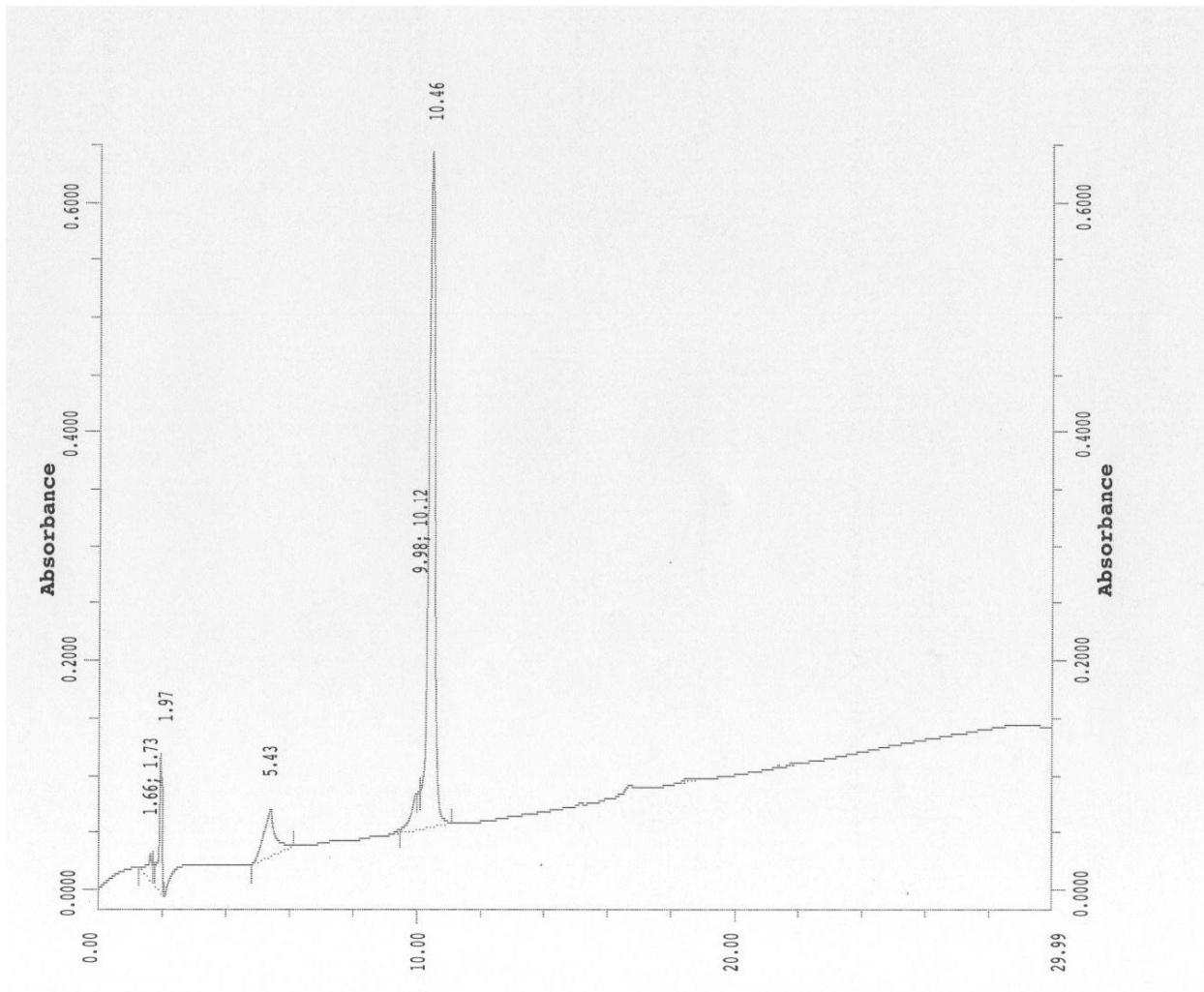
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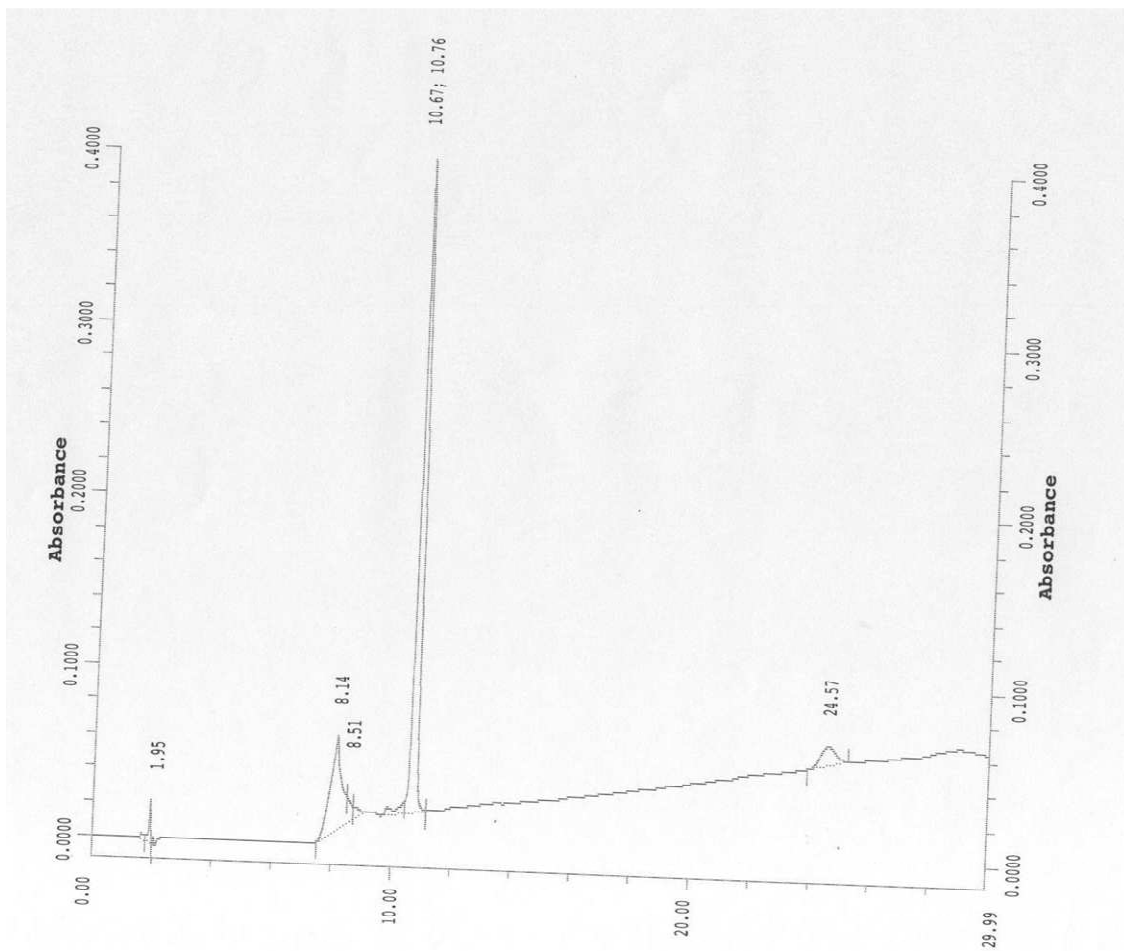
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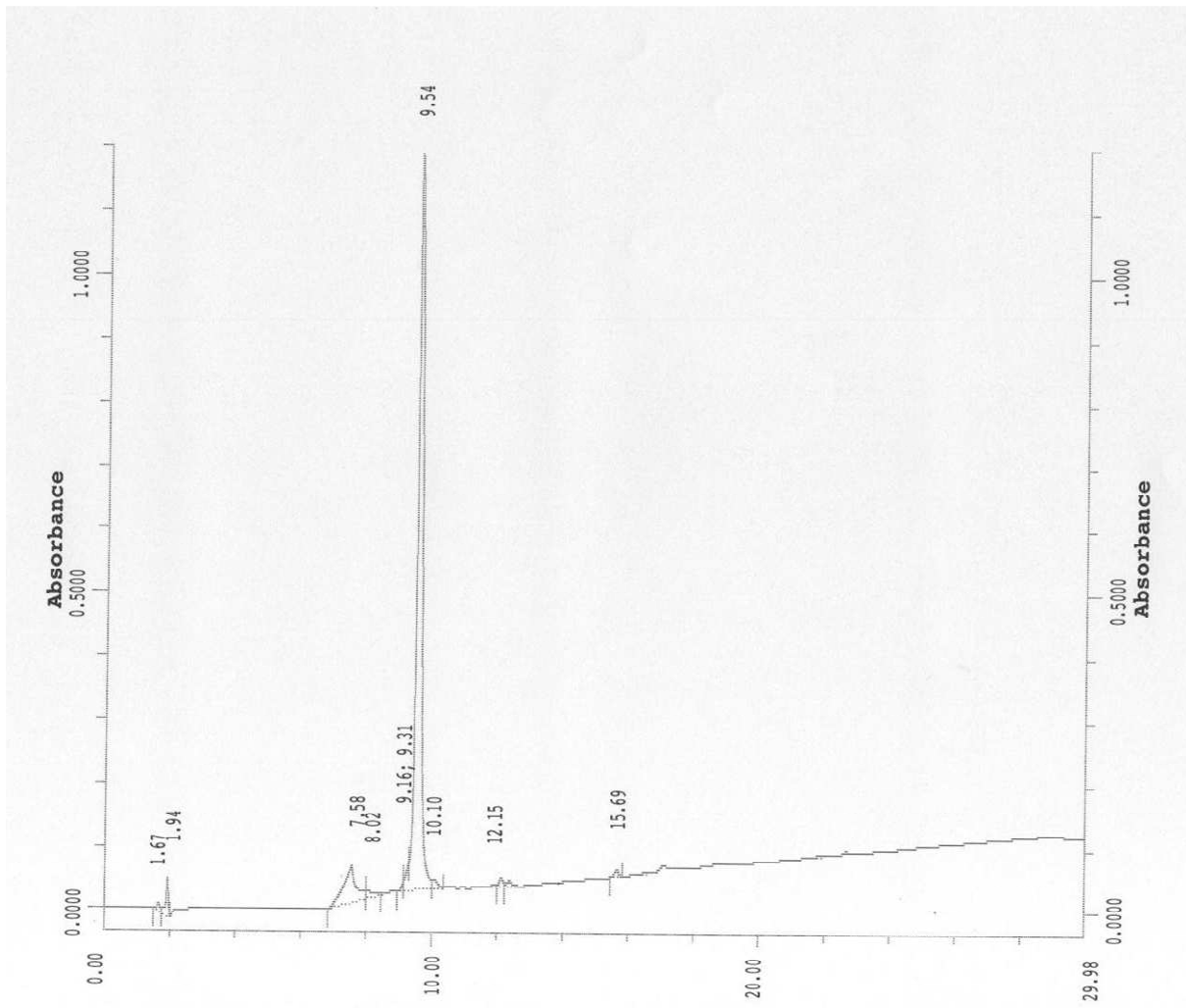
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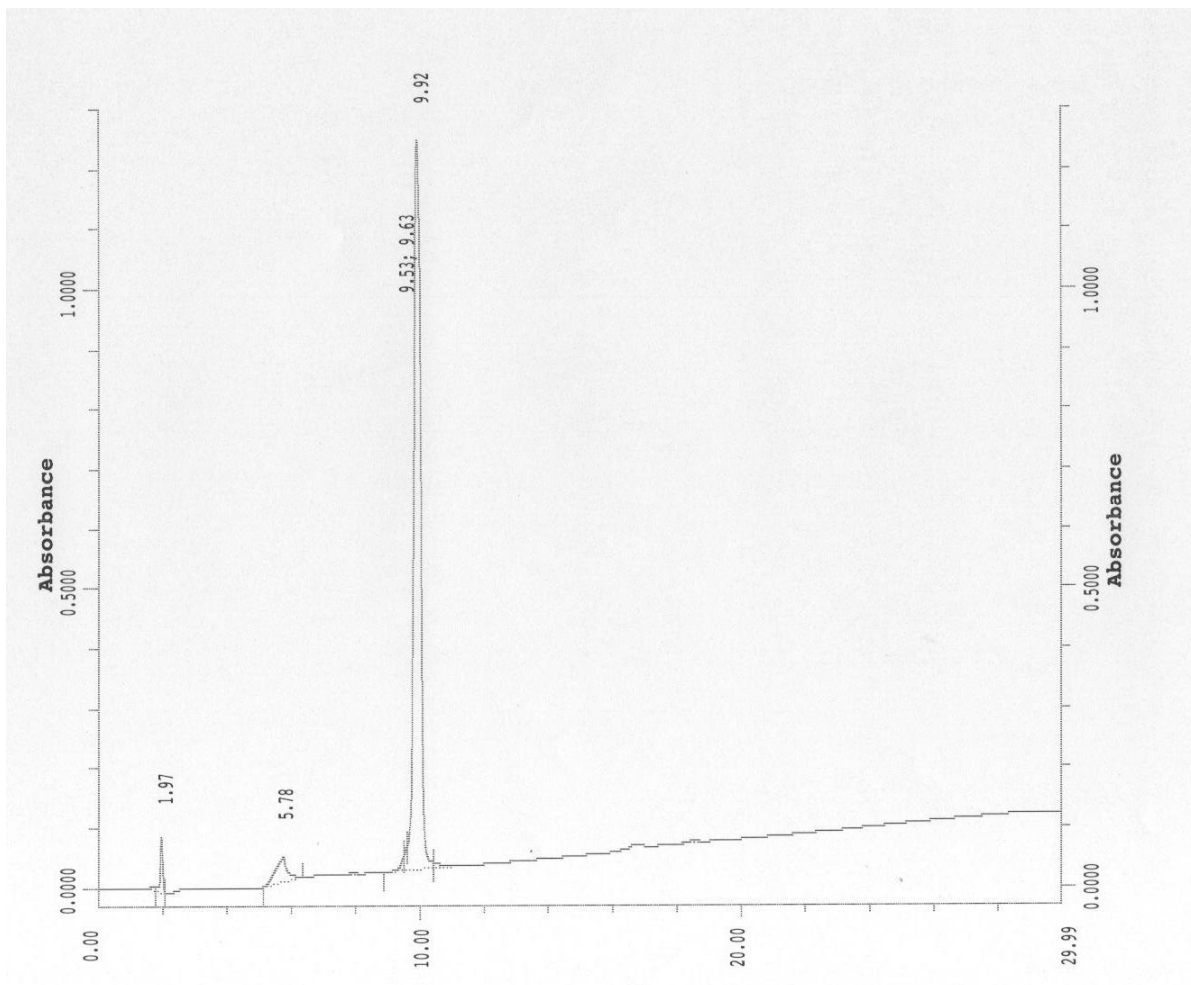
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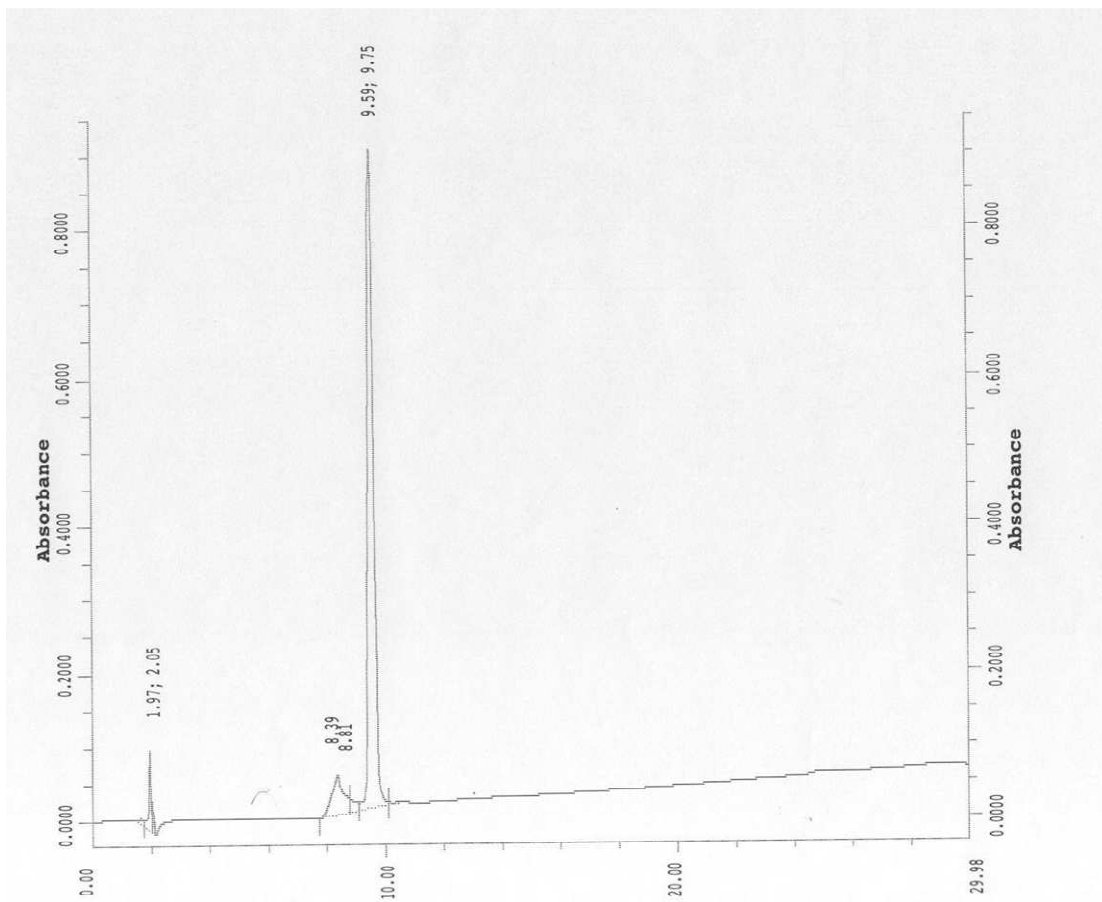
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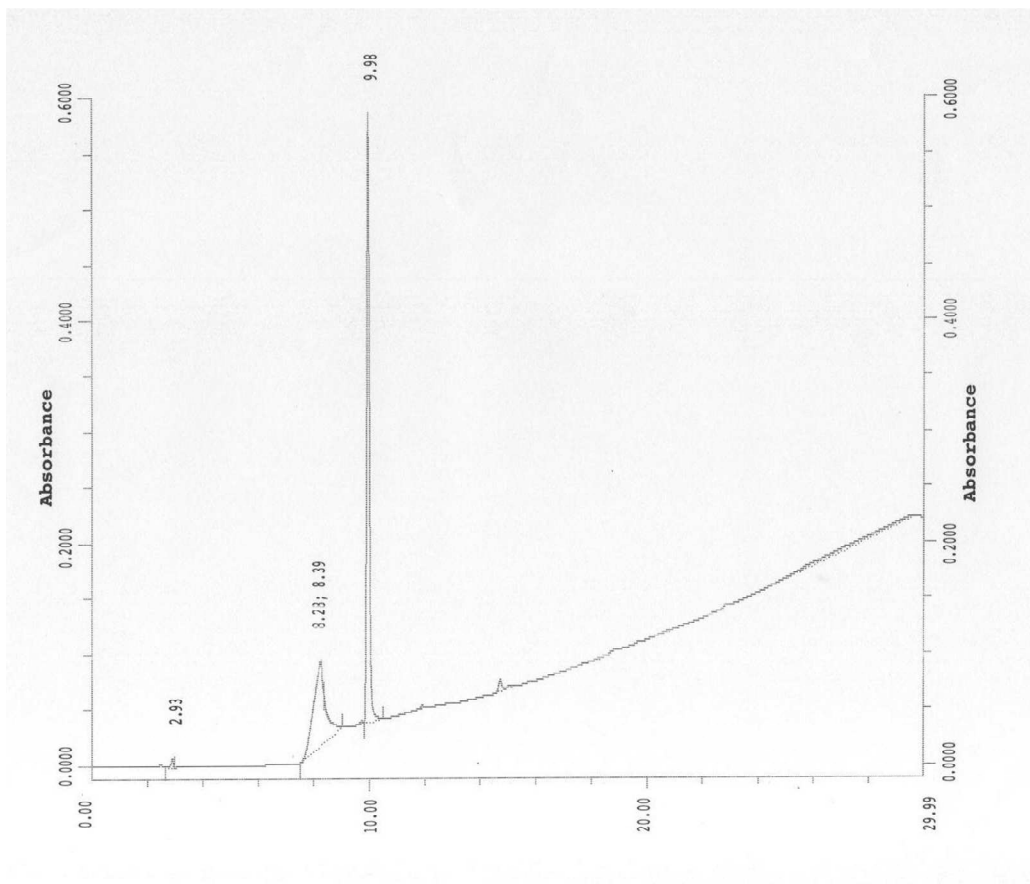
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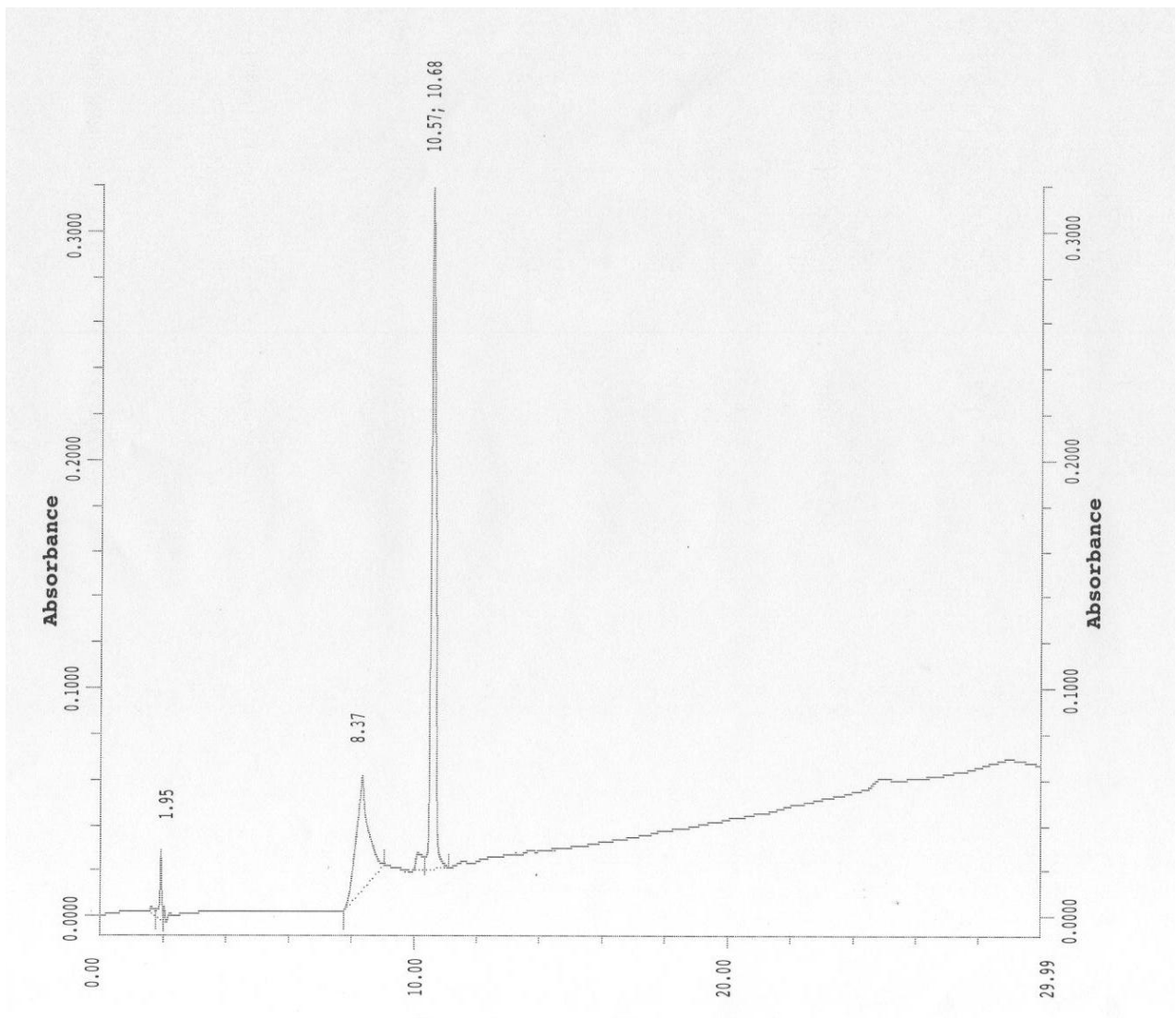
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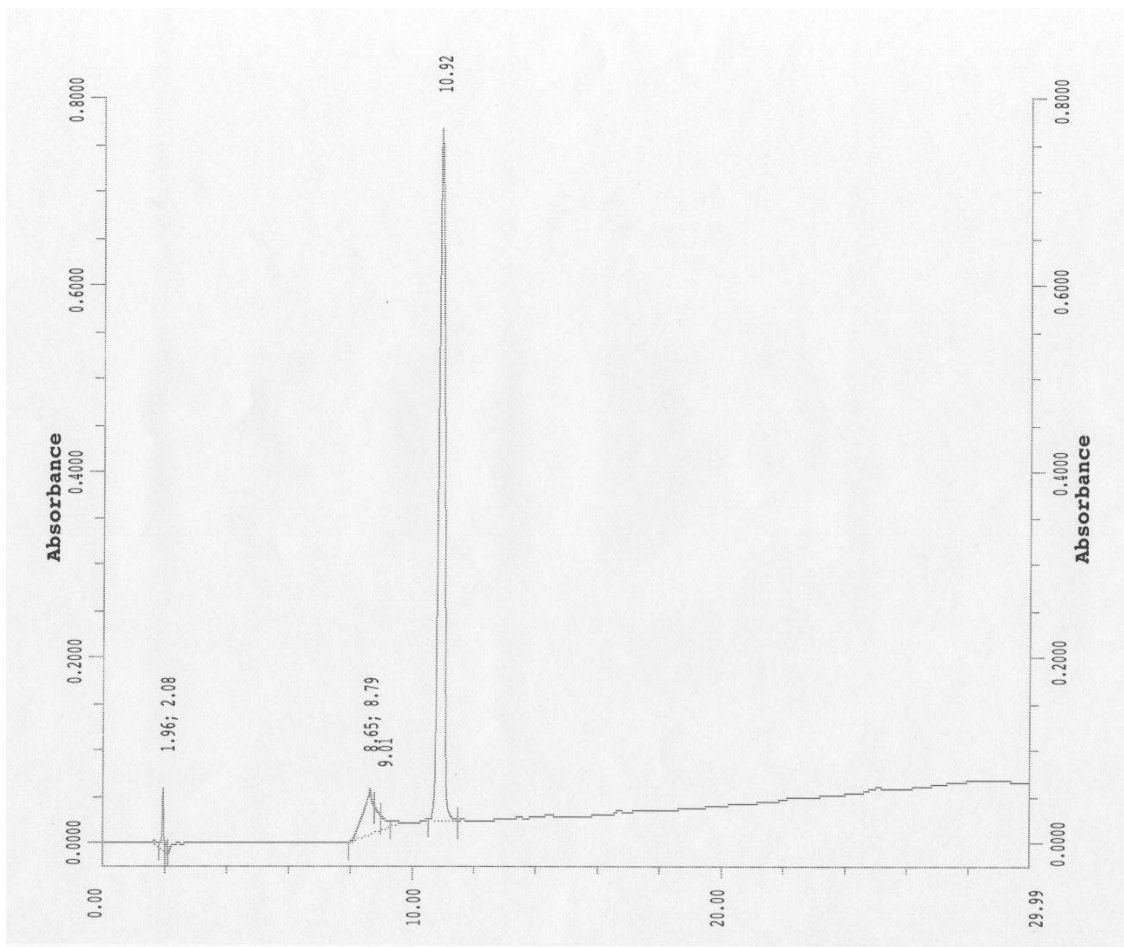
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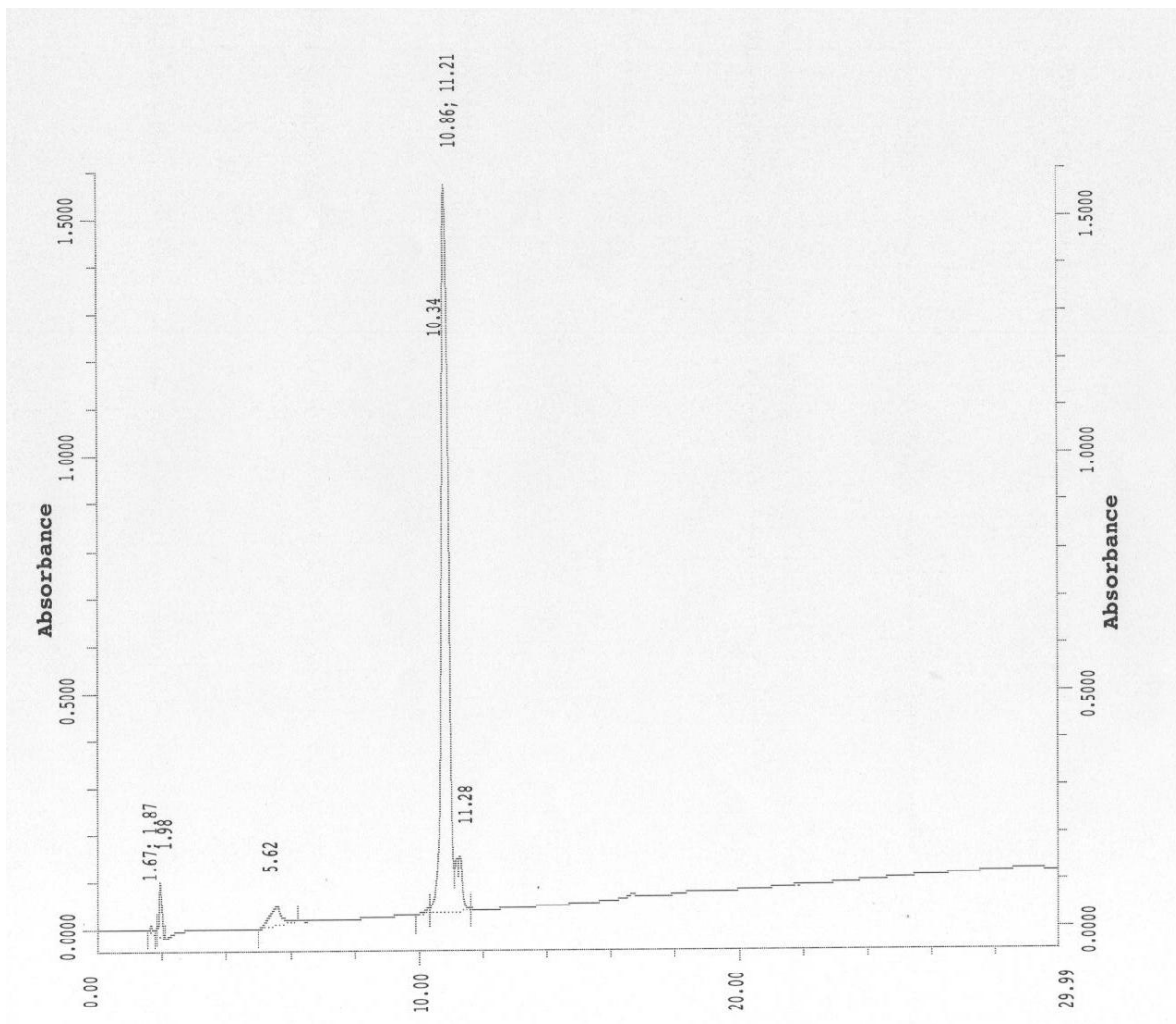
Compound 12



Compound 13

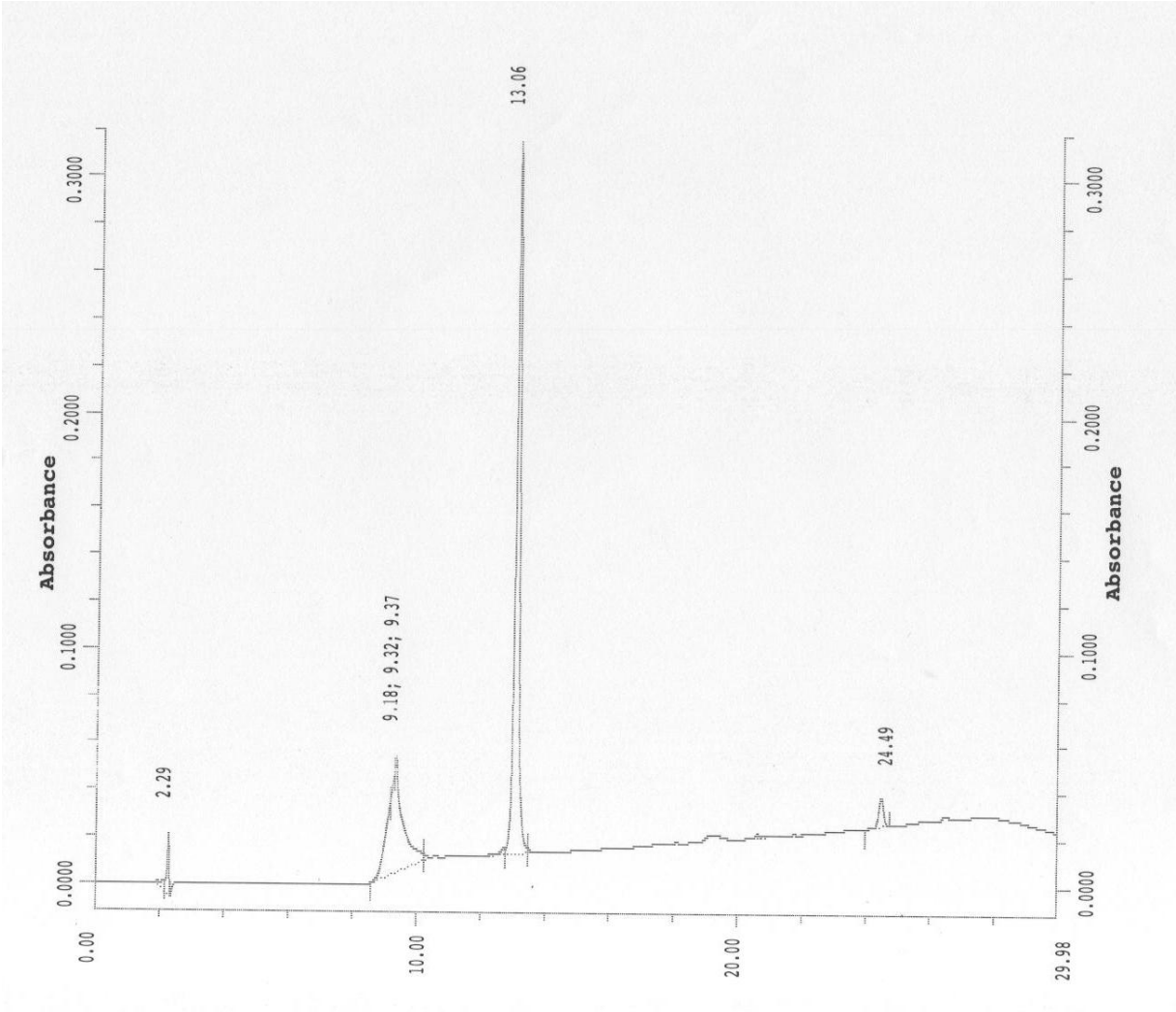


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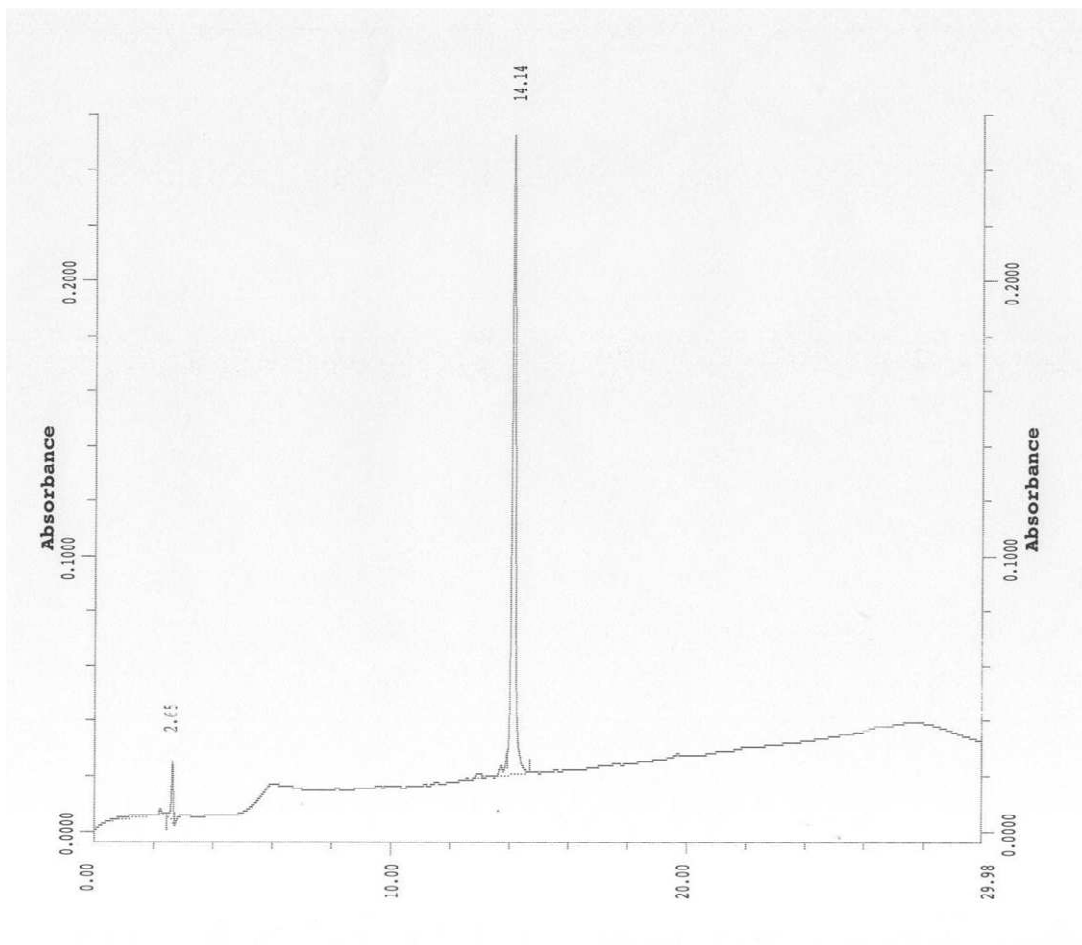


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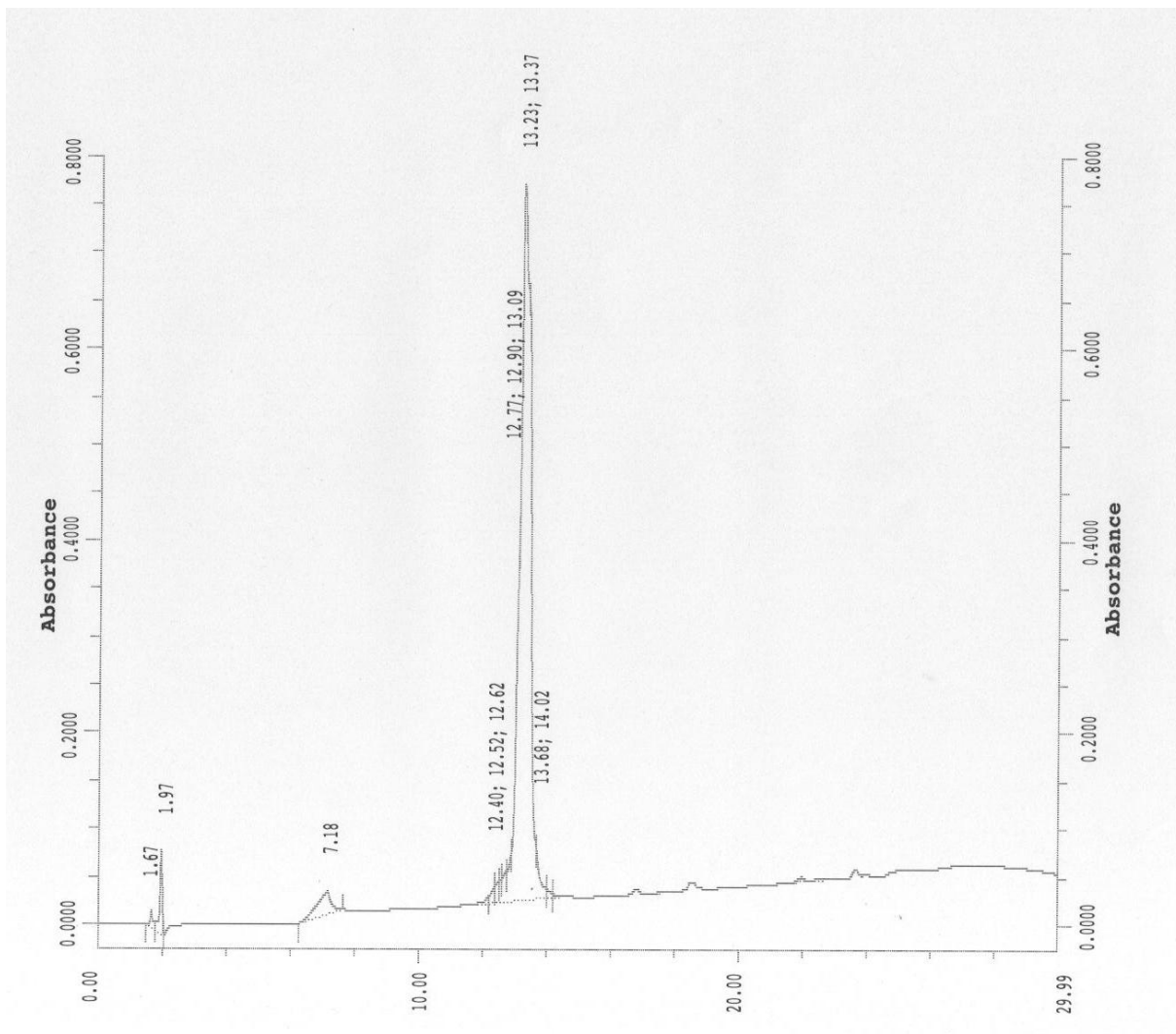
HPLC chromatograms of hNPS analogues (system II)



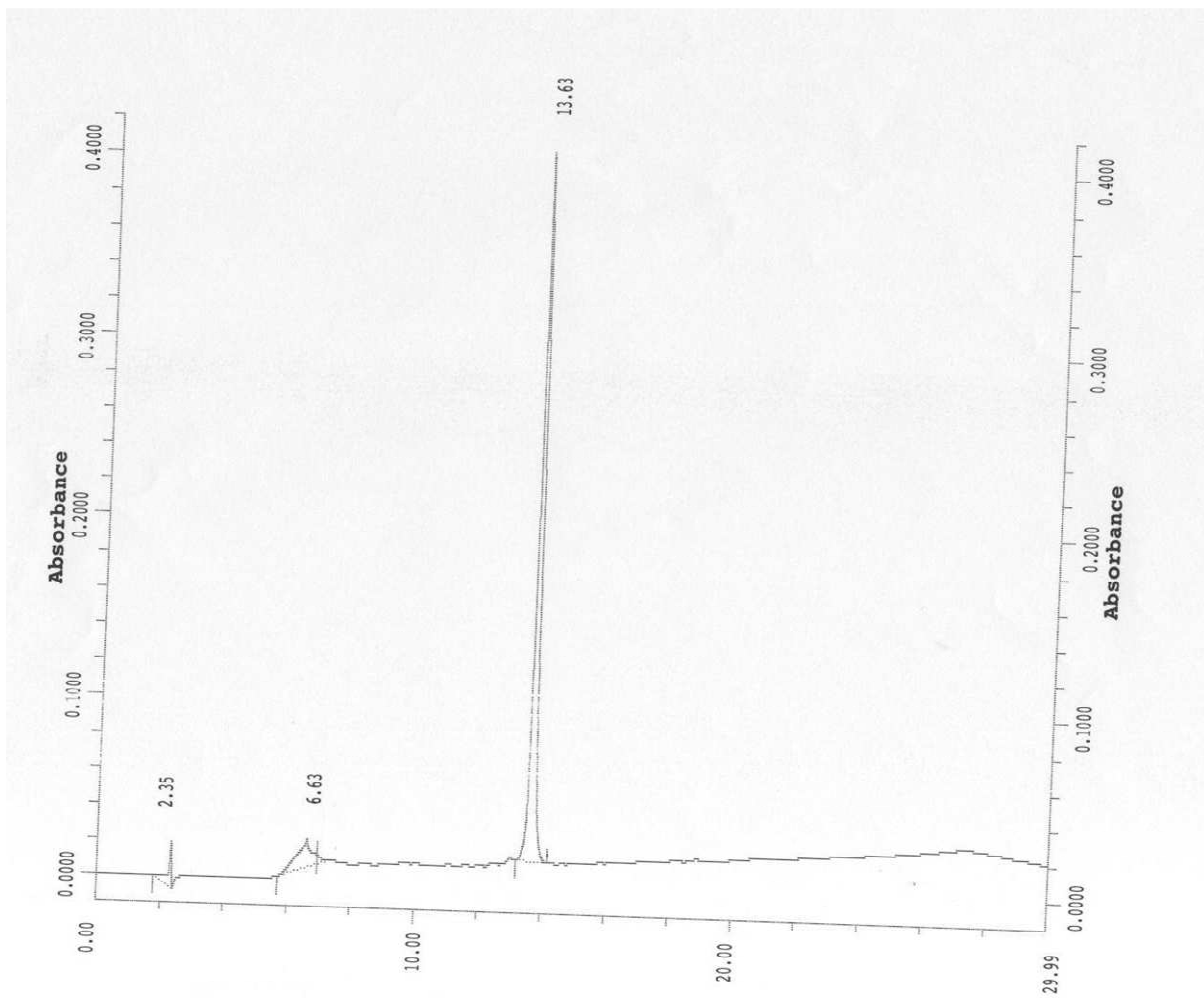
hNPS



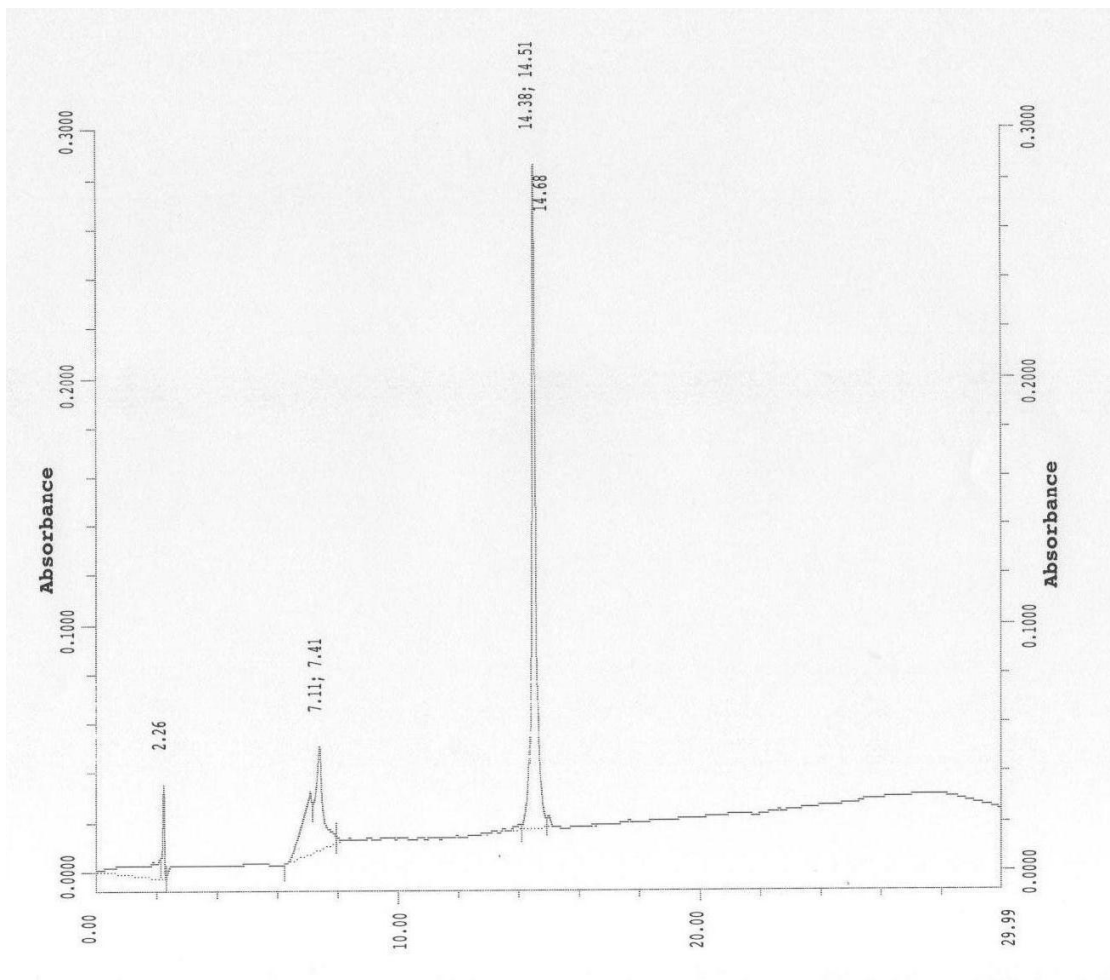
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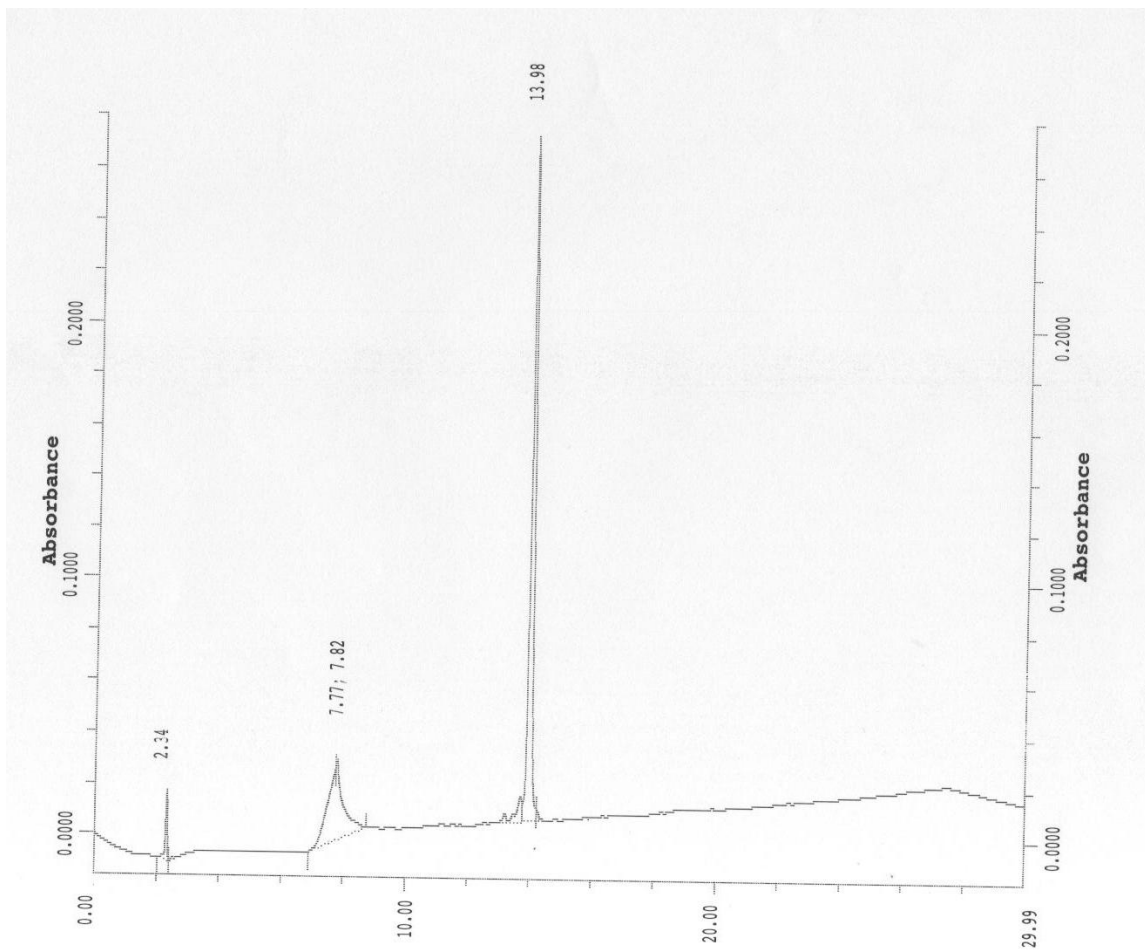
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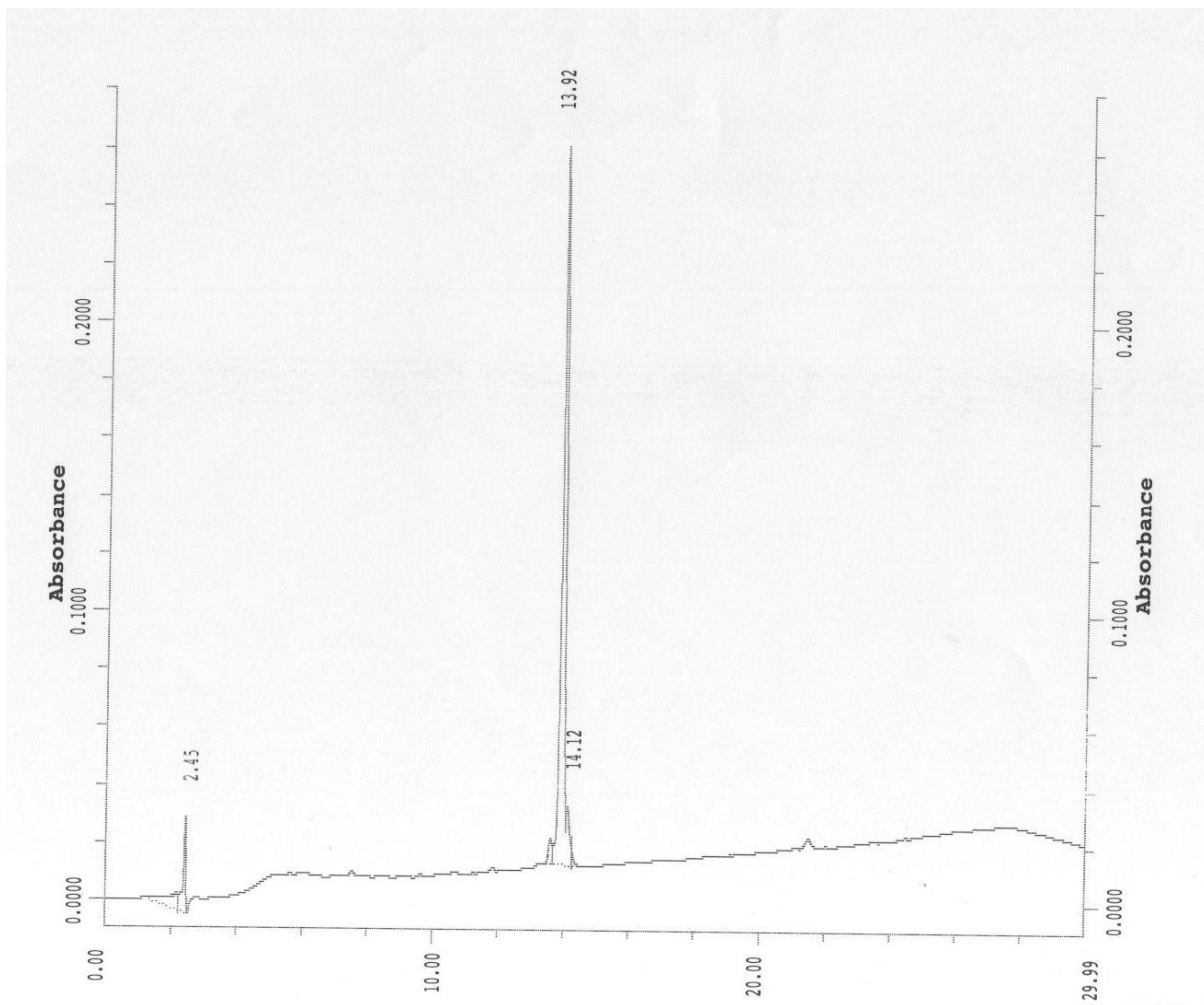
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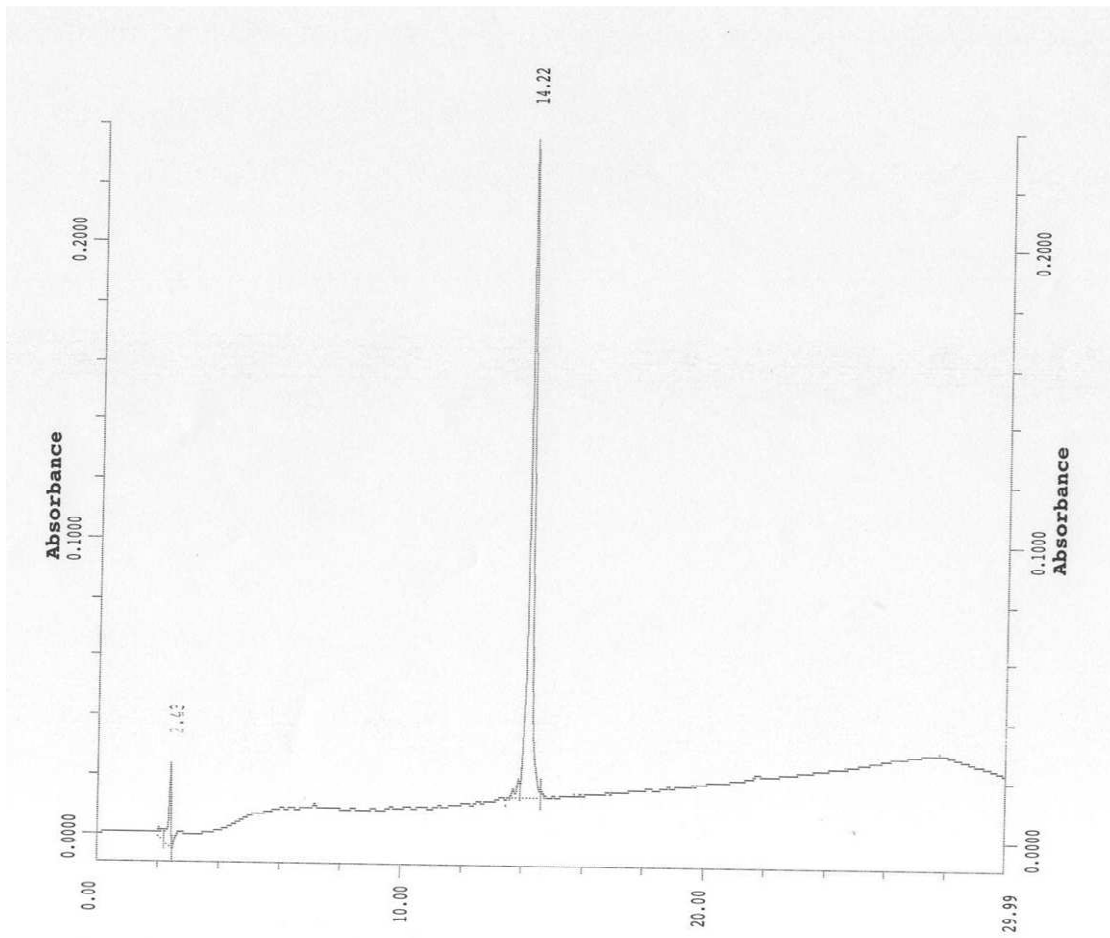
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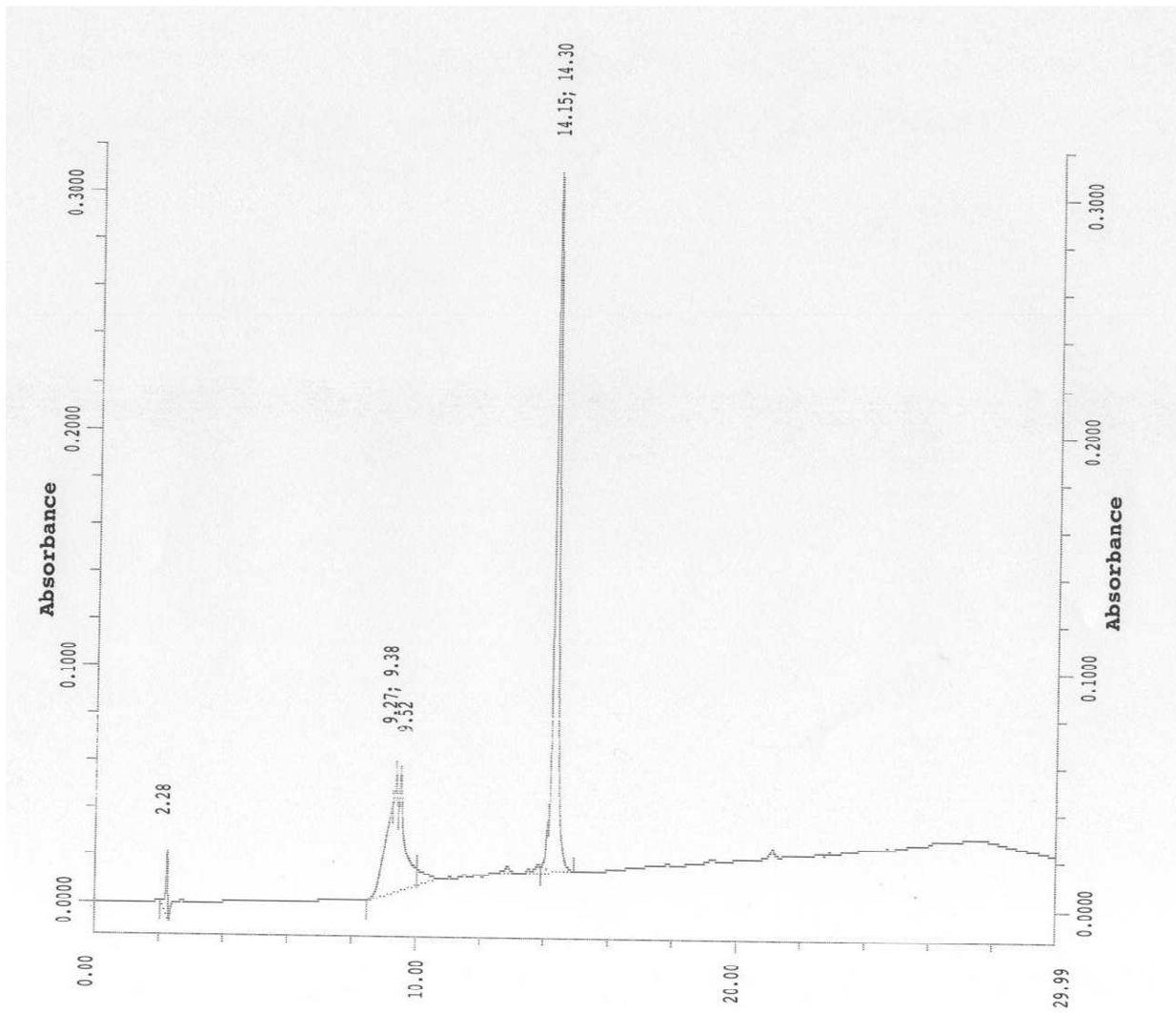
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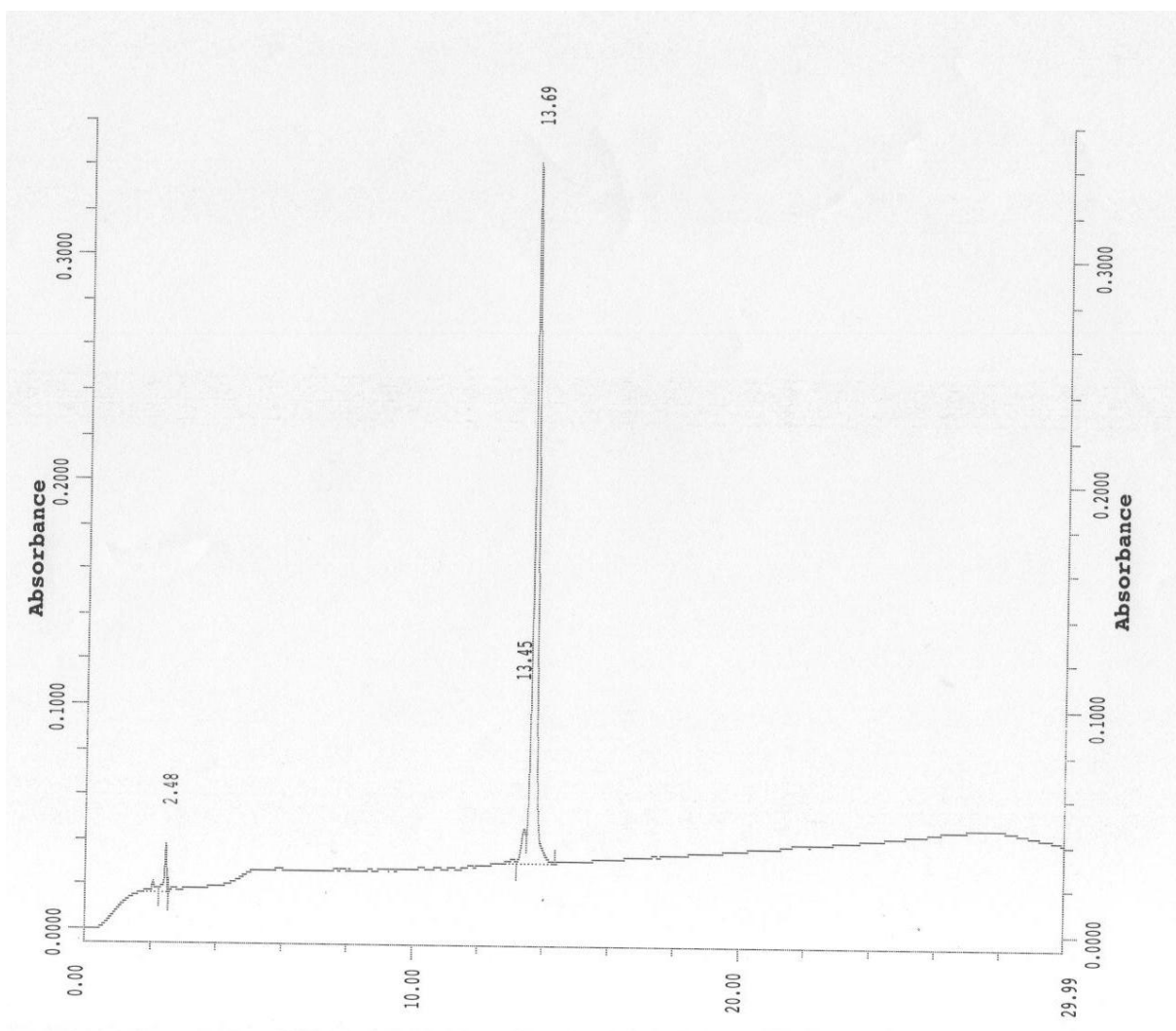
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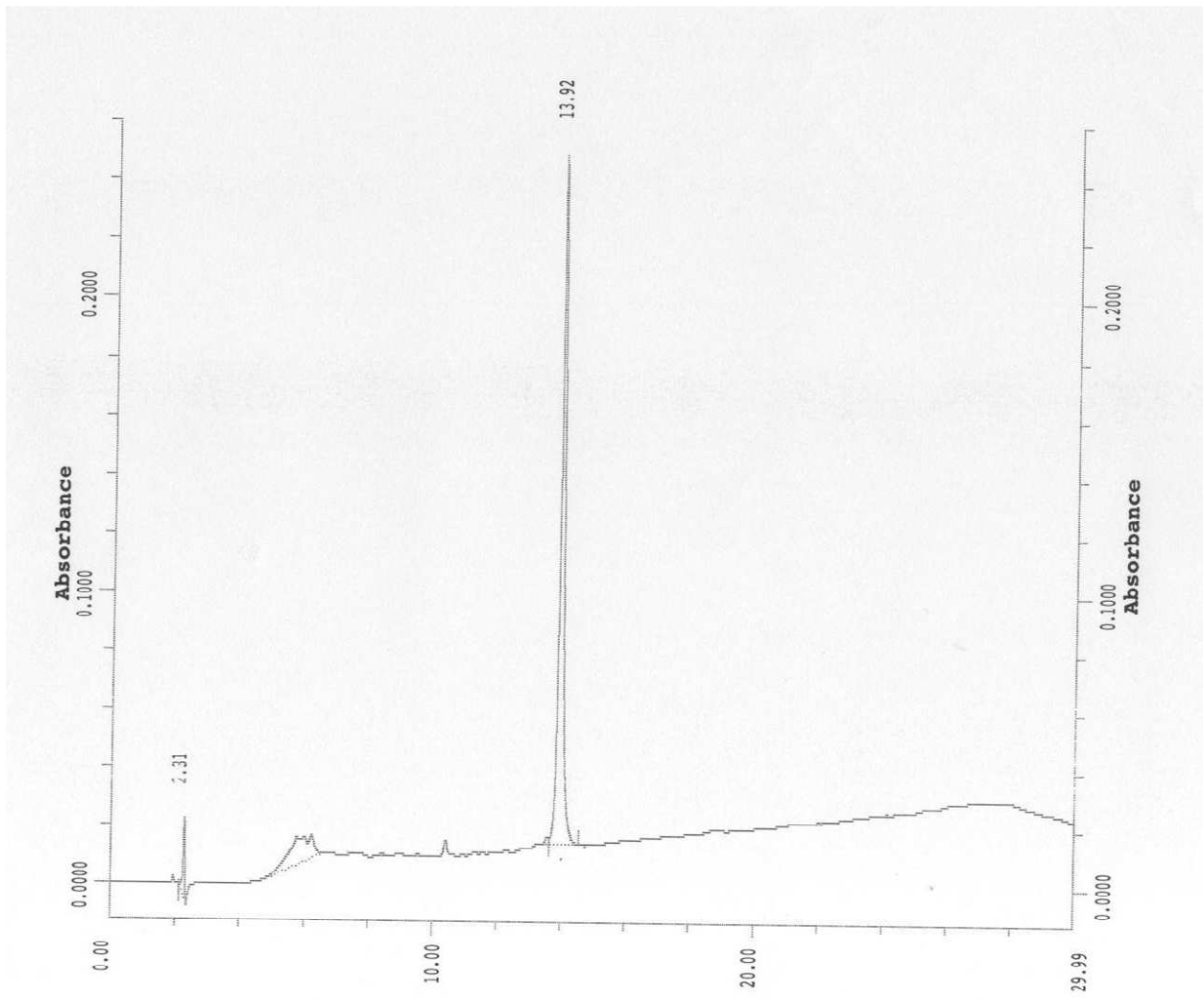
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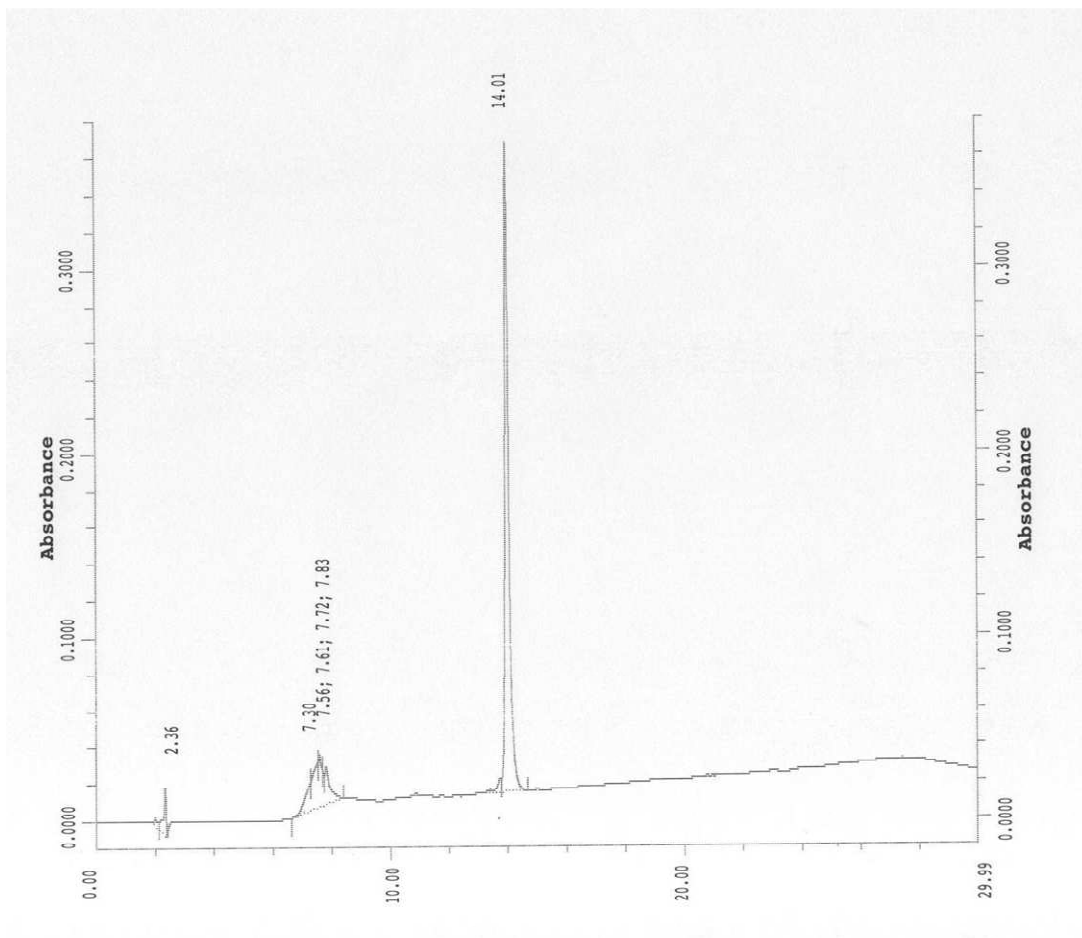
Compound 8



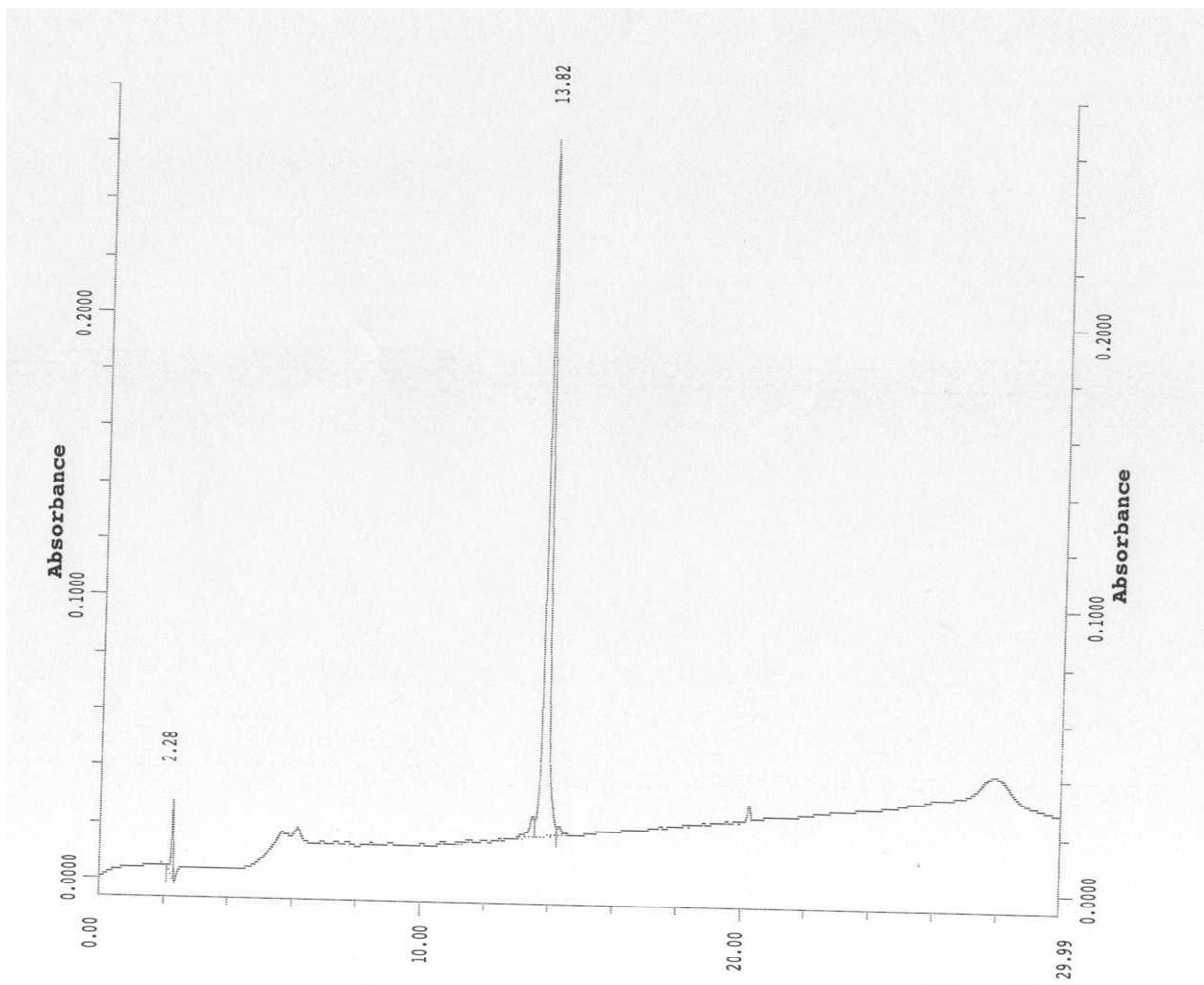
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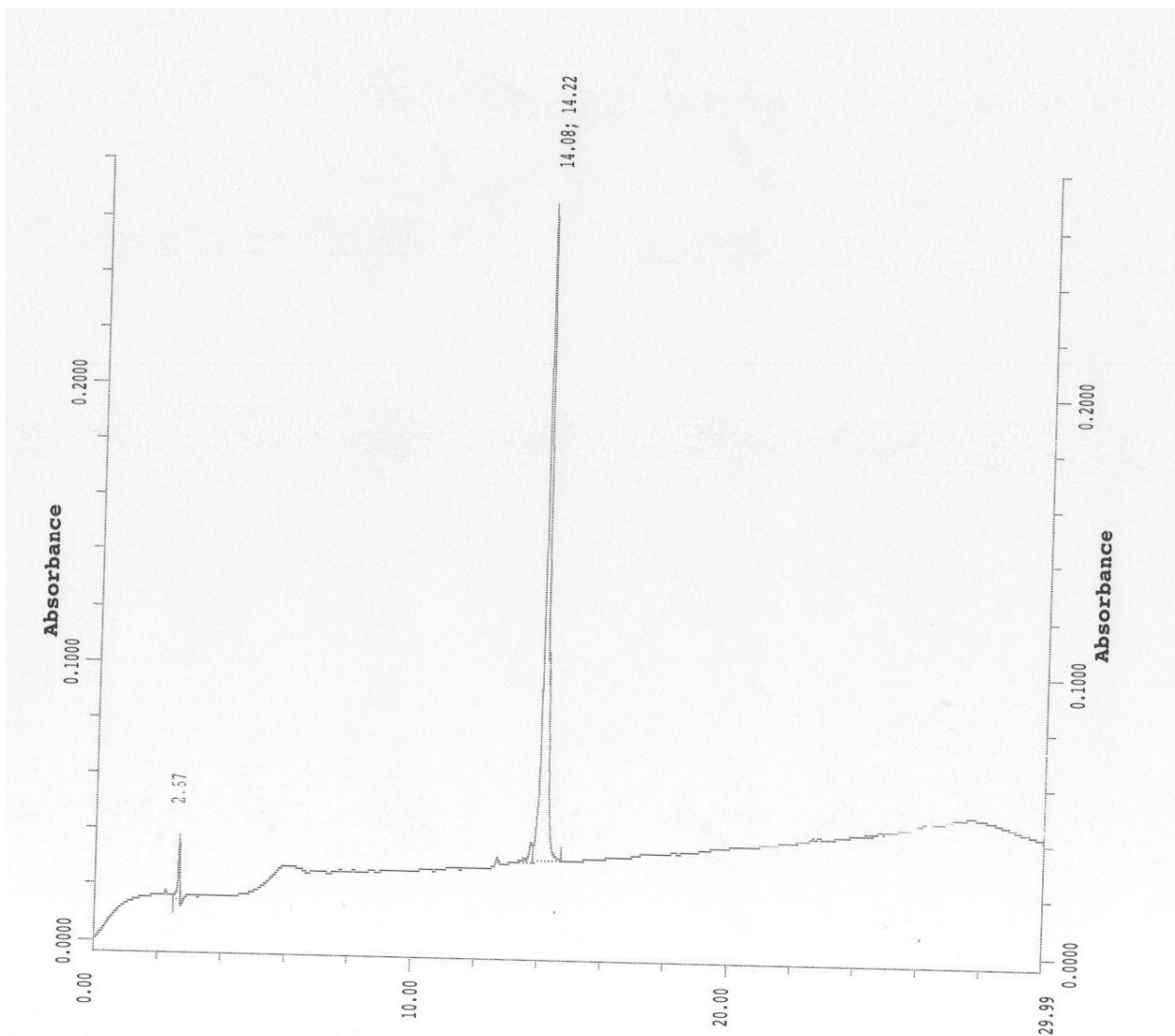
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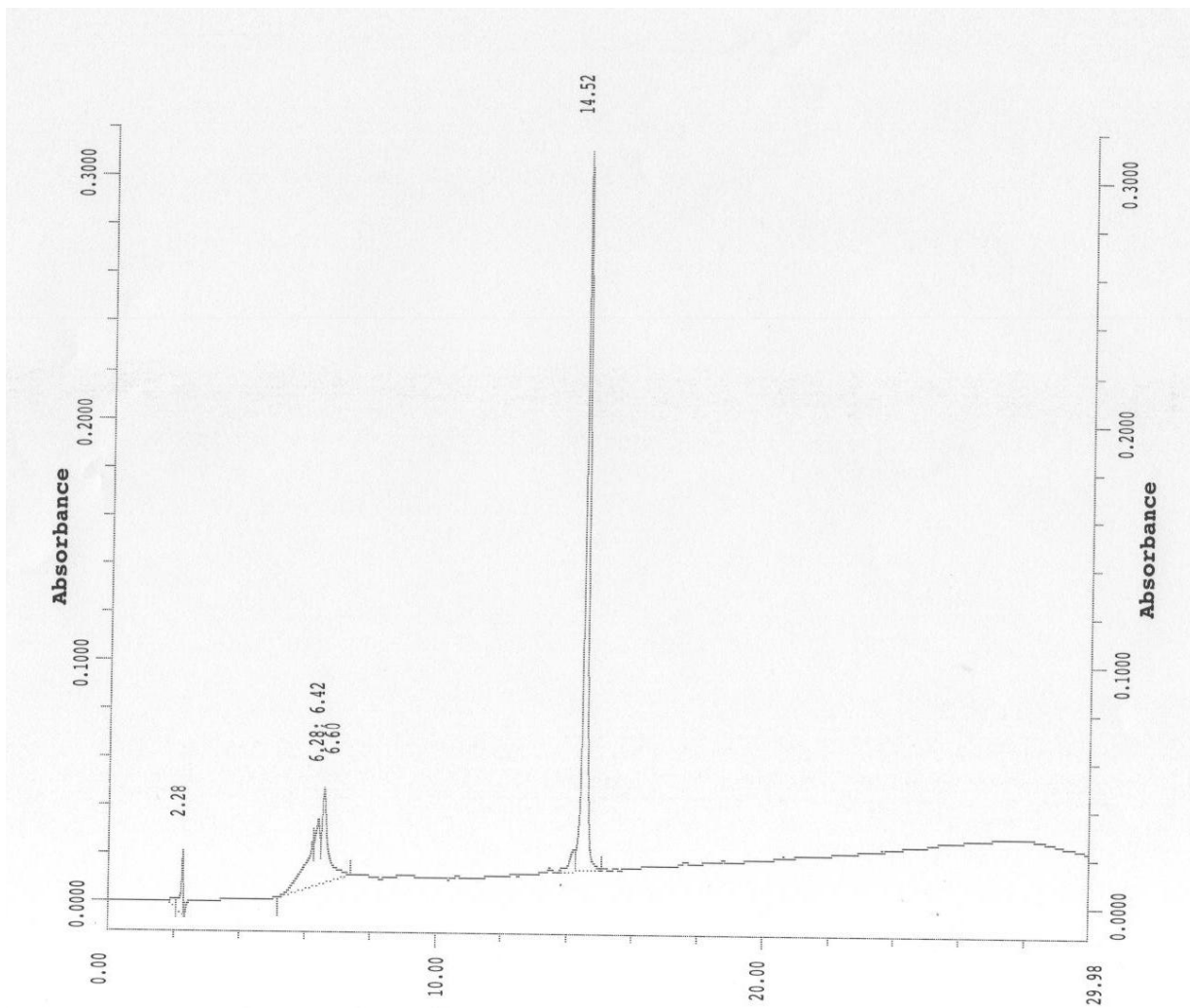
Compound 11



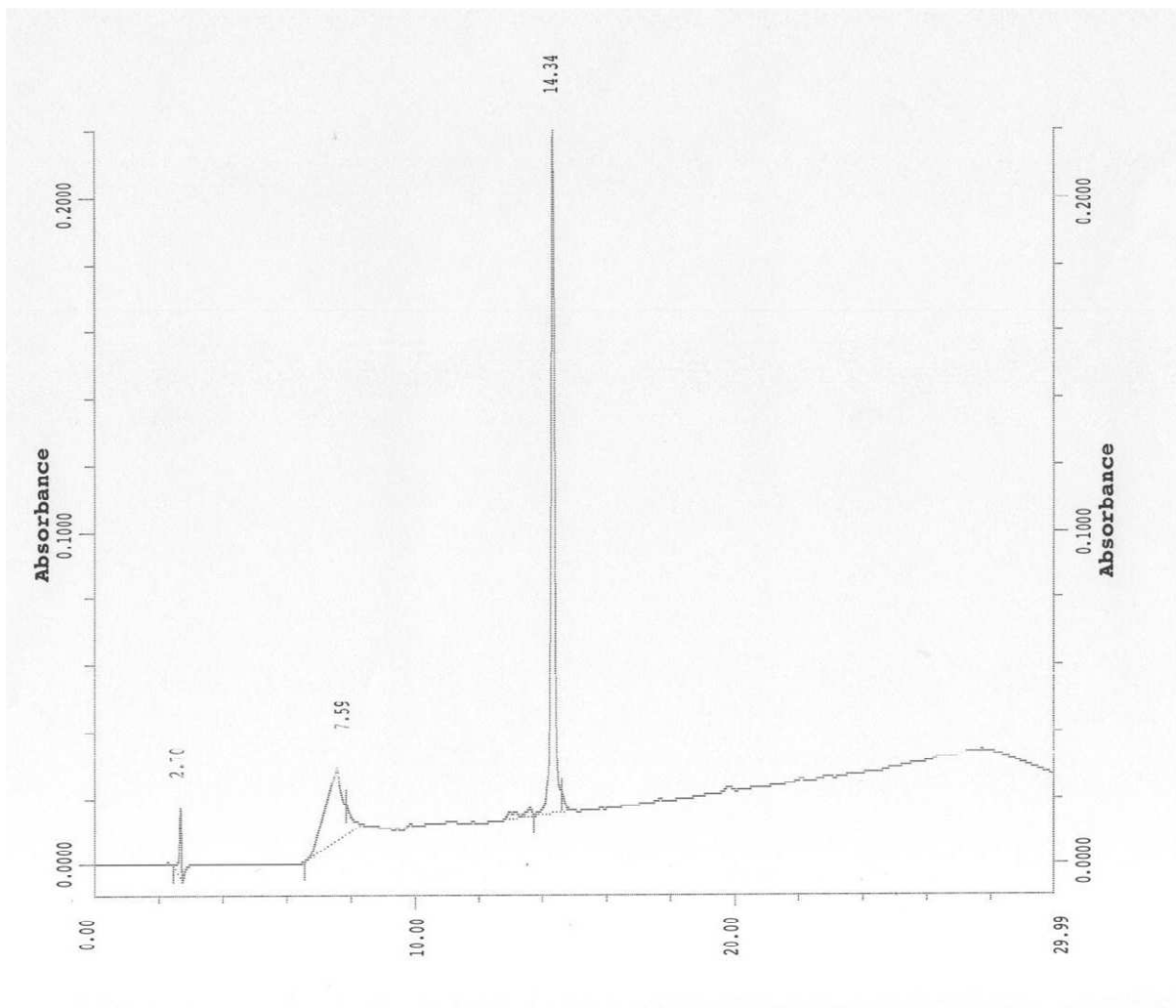
Compound 12



Compound 13



Compound 14



Compound 15