Development of test method for determination of chicory content in coffee-chicory powder

Project Code- FAD 0011

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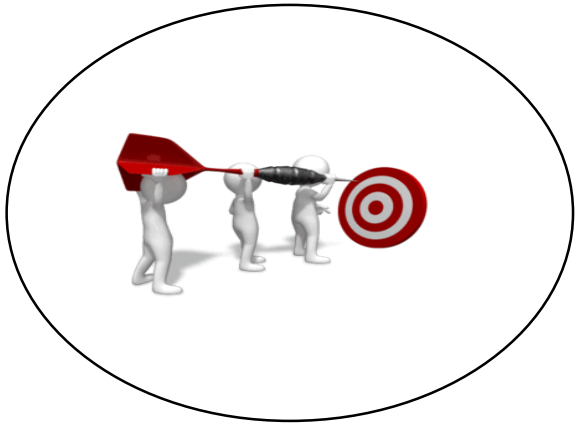
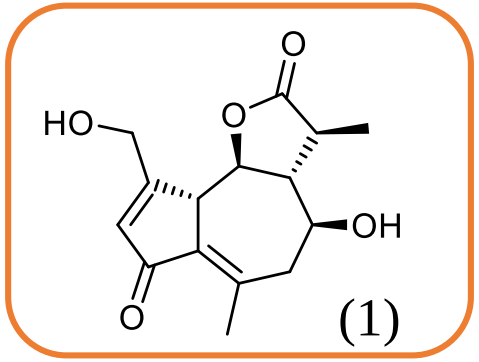
Objectives

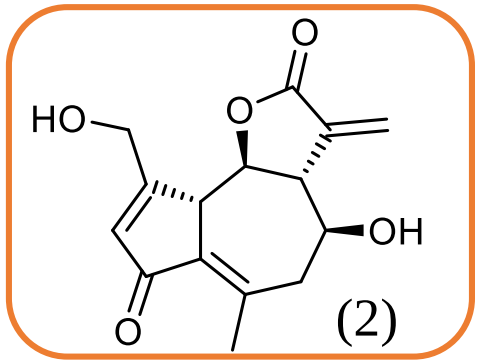
- To extract caffeine and chicoric acid from the commercially available samples using an ultrasound-assisted sonication technique
- To determine the amount of caffeine and chicoric acid in the sample using a high-performance liquid chromatography (HPLC) method
- The developed HPLC method will be validated and then adapted to determine the amount of chicoric acid and caffeine in commercially available samples



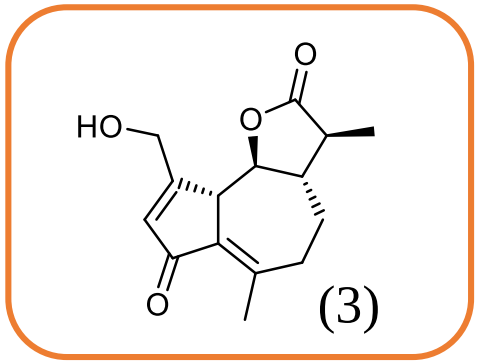
Literature Review

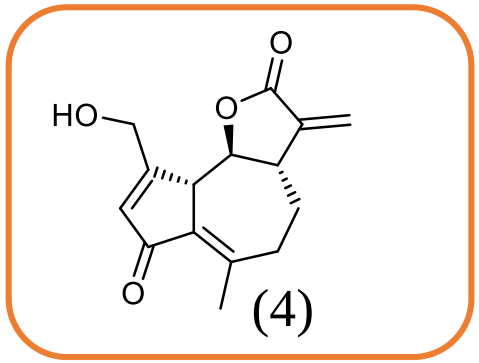
Major sesquiterpene lactones of chicory root

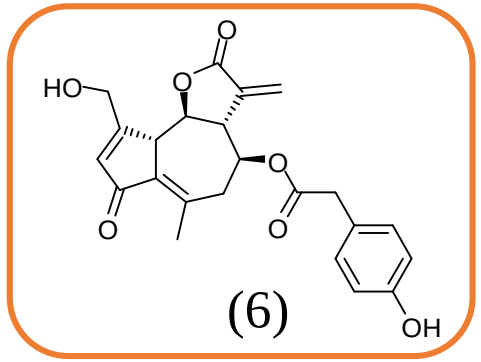


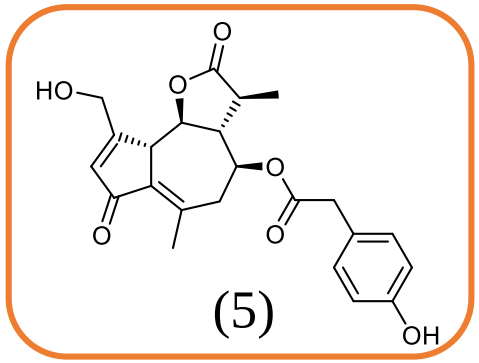


1) 11(S),13-dihydrolactucin
2) lactucin
3) 11(S),13-dihydro-8-deoxylactucin
4) 8-deoxylactucin
5) 11(S),13-dihydrolactucopicrin
6) lactucopicrin







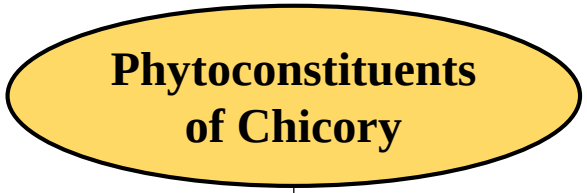


Hakkinen, S.T et.al., 2021. Chicory extracts and sesquiterpene lactones show potent activity against bacterial and fungal pathogens *Pharmaceuticals*, *14*(9), p.941.

Methods reported for Sesquiterpene lactones analysis from chicory roots

Source	Sesquiterpene lactones						Extraction method	Analytical method used	Reference
	(1) µg/g	(2) µg/g	(3) µg/g	(4) µg/g	(5) µg/g	(6) µg/g			
Chicory Roots	53.3 ± 3.3	97.4 ± 5.5	42.4 ± 1.2	6.8 ± 1.3	20.9 ± 0.6	4.7 ± 1.5	Maceration	HPLC/DAD	Honorine Willeman, 2014
Chicory Flour	147.8± 7.3	150 ± 0.1	318.8 ± 29.7	38.3 ± 6.5	27.2 ± 3.8	110.4 ± 11.8			
Chicory Roasted grains	1.3± 2.3	22.9 ± 0.1	92.8 ± 7.9	16.4 ± 5.4	1.3 ± 0.009	25.7 ± 3.3			
Freeze dried chicory root powder	642.3 ± 76.3 mg/kg	175.3 ± 32.9 mg /kg	-	-	-	-	Maceration-17hrs with agitation	UPLC DAD, LC-QTOF	Ruggieri, F., 2023
Chicory root powder	170.19 µg/mg	257.59 µg/mg	-	-	55.62 µg/mg	271.04 µg/mg	Supercritical fluid extraction	-	Suvi T. Häkkinen 2021
Chicory root	39.0 mg	63.2 mg	36.0 mg	39.3 mg	16.5 mg	7.8 mg	Maceration	UPLC-MS	Hang Fan 2017
Aerial parts	*	*	*	*	*	*		Orbitrap HRMS/ & LCMS/MS	Graziani, G., 2015.

1) 11(S),13-dihydrolactucin, 2) lactucin 3)11(S),13-dihydro-8-deoxylactucin 4) 8-deoxylactucin 5) 11(S),13dihydrolactucopicrin 6) lactucopicrin
Note- * method of preparation for lactone-rich extract



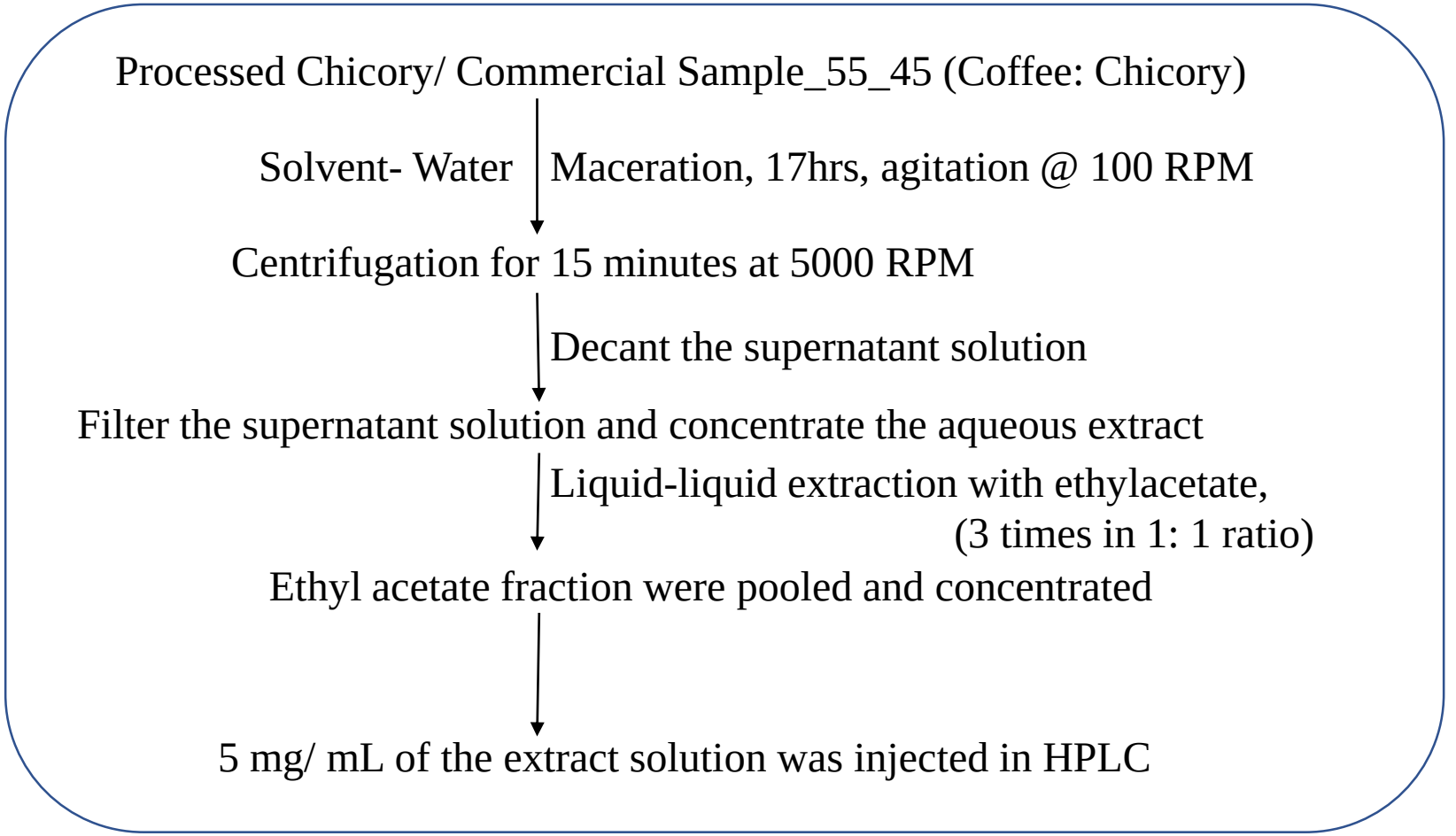
Caffeic acid
Quinic acid
Caffeoylquinic acid
Caftaric acid
Quercetin
Kaempferol
Caffeoylquinic acid
Dicaffeoylquinic acid
Chicoric acid
Cinnamic acid
Caftaric acid
Hydroxycinnamic acid
Oxalic, Succinic, Shikimic and Quinic acids

8-Deoxylactucin
13-dihydrolactucin
Jacquinelin
Crepidiaside B
Lactucin
Lactucopicrin
Magnolialide
Ixerisoside D
Loliolide
3,4β-Dihydro-15-dehydrolactucopicrin

Apigenin-7-O-glucoside
Chrysoeriol-3-O-glucoside
Dicaffeoylquinic acid
Myricetin-7-O-(6-O-malonyl)-glucoside
Dimethoxy cinnamoyl shikimic acid
Kaempferol-3-O-sophorosid
Isorhamnetin-7-O-glucoside

Singh, R., 2018. *Cichorium intybus* L: A review on phytochemistry and pharmacology. *International Journal of Chemical Studies*, *6*(3), pp.1272-1280.

Extraction Method for Lactone analysis



Extraction: Ruggieri, F.et.al., 2023.A Three-Step Process to Isolate Large Quantities of Bioactive Sesquiterpene Lactones from *Cichorium intybus* L. Roots and Semisynthesis of Chicory STLs Standards. *Pharmaceuticals*, *16*(5), p.771.

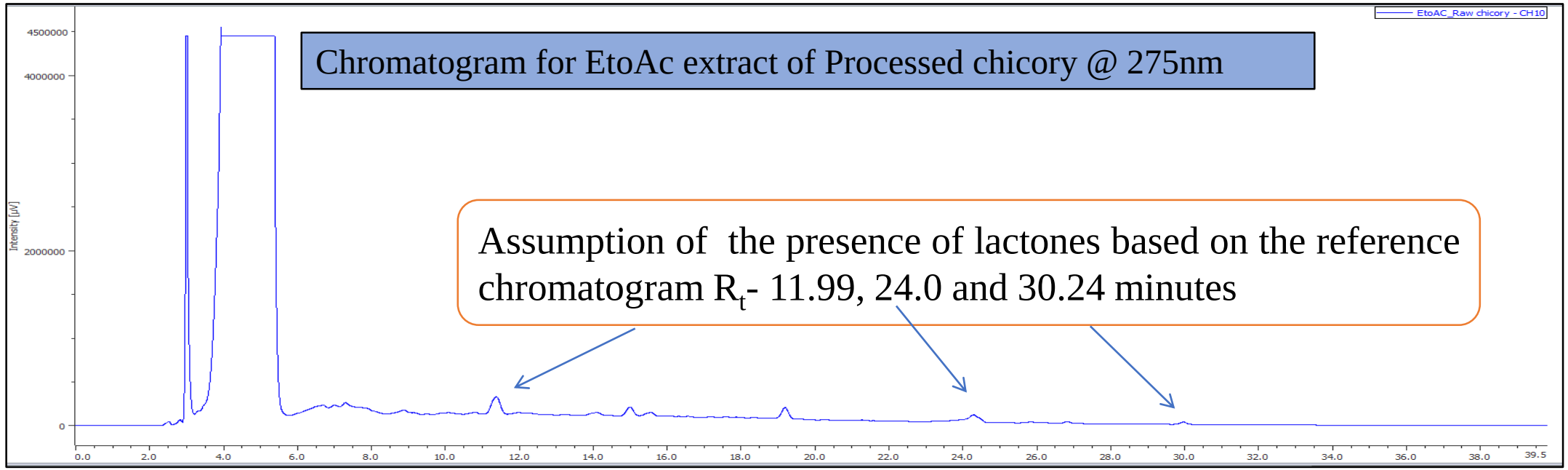
HPLC conditions

- ✓ **Column:** Waters X-Bridge C18 (250 X 4.6 mm, 5 µm)
- ✓ **Flow rate**: 1.0 mL/min
- ✓ **Column temperature:** 30ºC
- ✓ **Mobile phase A:** Water
- ✓ **Mobile phase B:** Acetonitrile
- ✓ **Injection volume-** 20 µL
- ✓ **Run time:** 40 minutes
- ✓ **Elution mode:** Gradient

Time (mins)	Mobile Phase A (%)	Mobile Phase B (%)
0.05	90.0	10.0
30	58.0	42.0
35	90.0	10.0
40	90.0	10.0

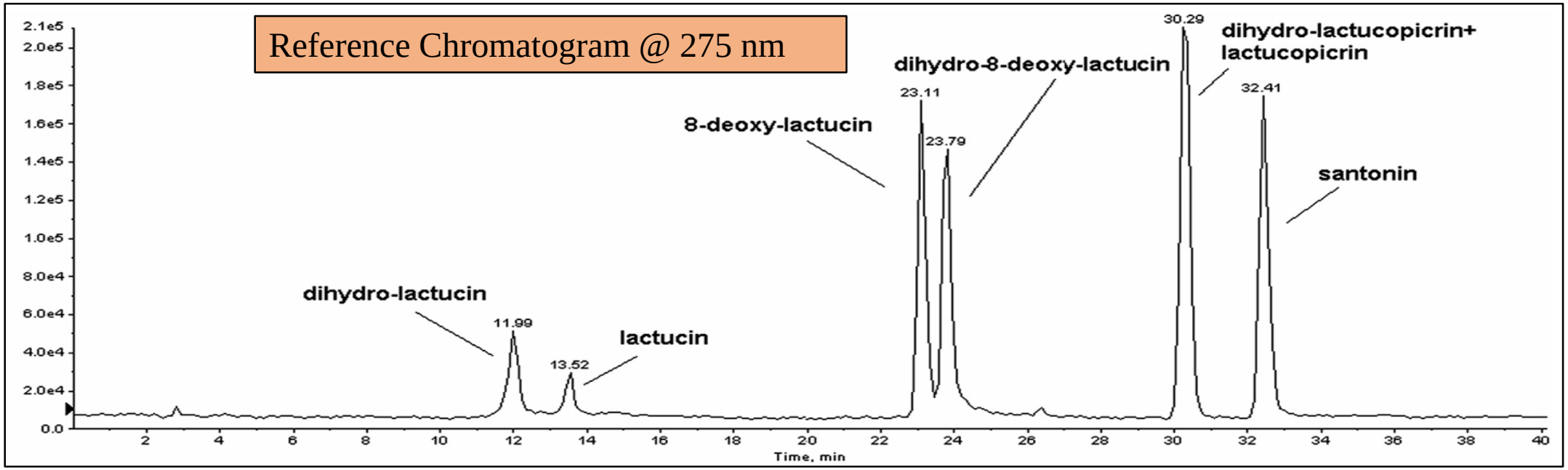
Graziani, G.et.al., 2015. Profiling chicory sesquiterpene lactones by high-resolution mass spectrometry. *Food Research International*, 67, pp.193-198

HPLC Chromatogram of Ethyl acetate extract of Processed chicory

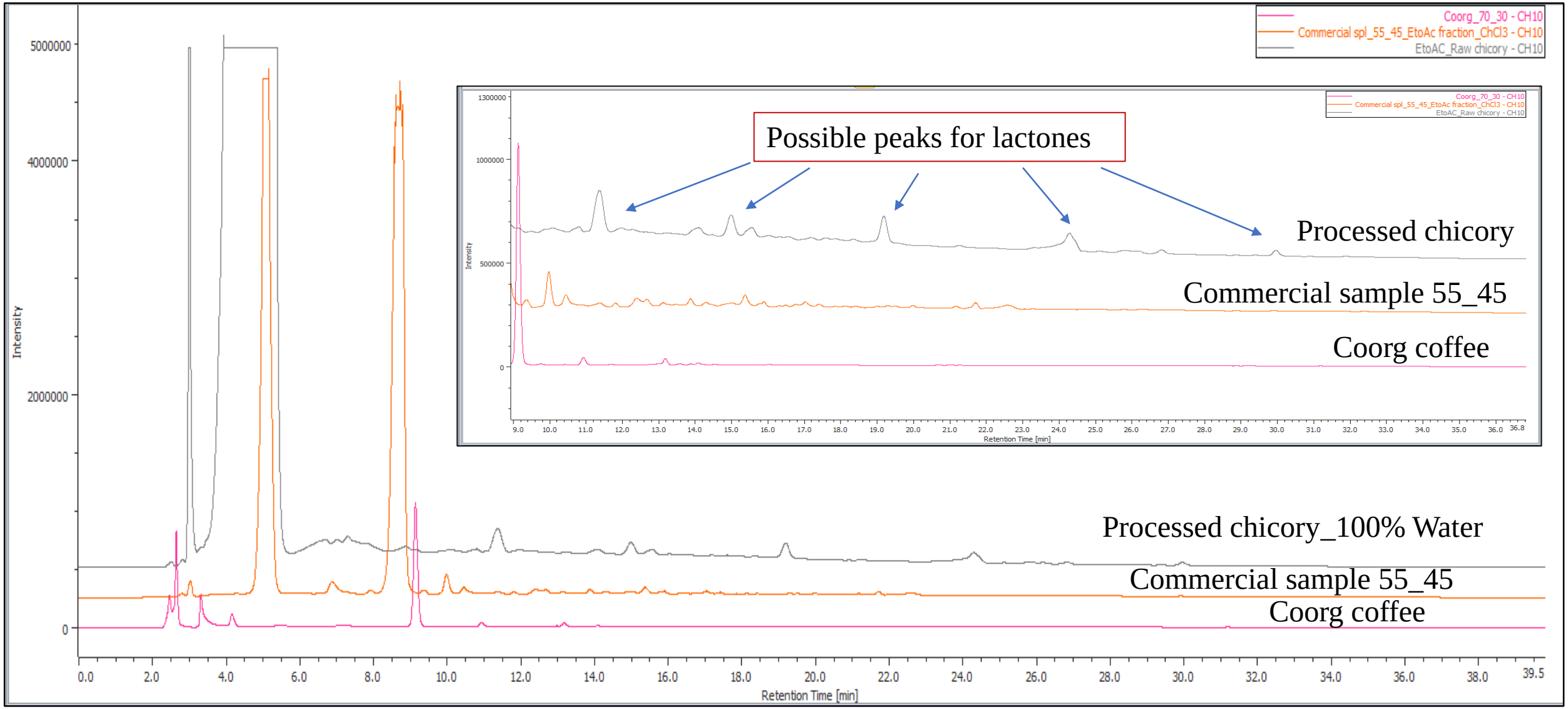


Chromatogram for EtoAc extract of Processed chicory @ 275nm

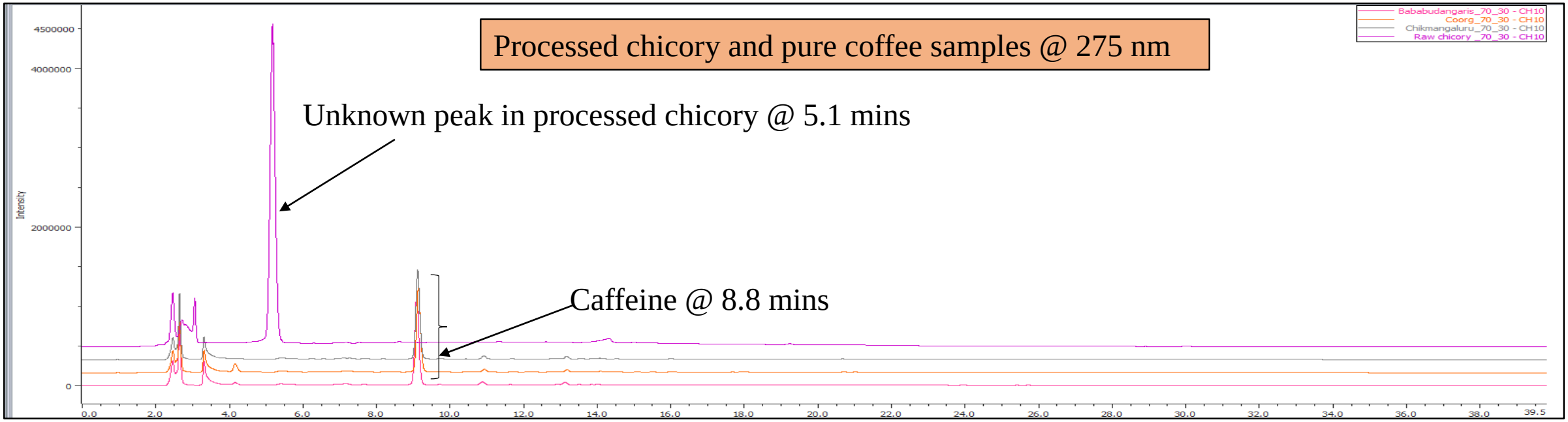
Assumption of the presence of lactones based on the reference chromatogram R_t- 11.99, 24.0 and 30.24 minutes

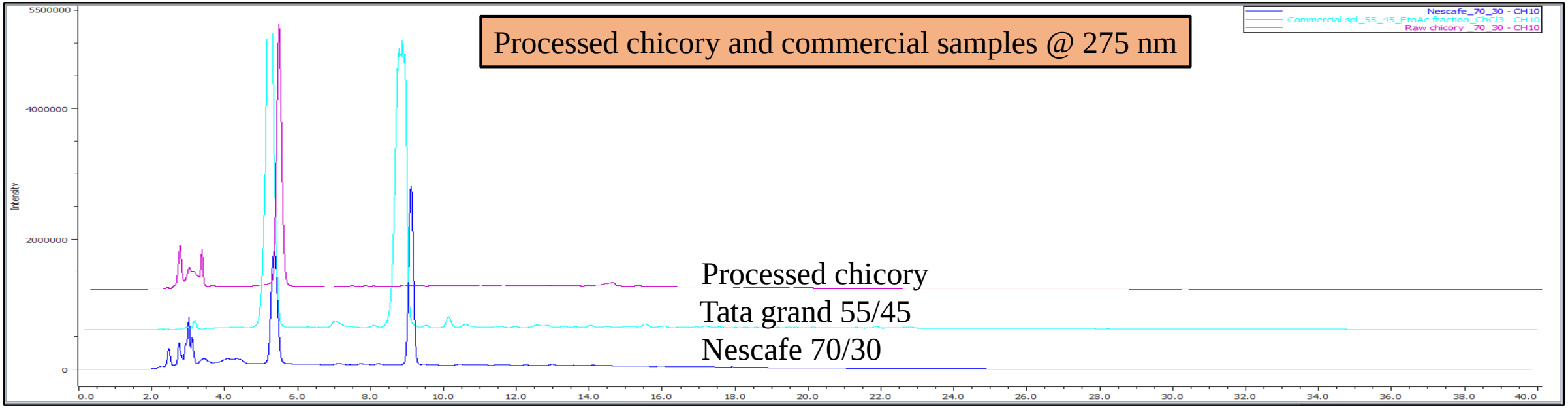


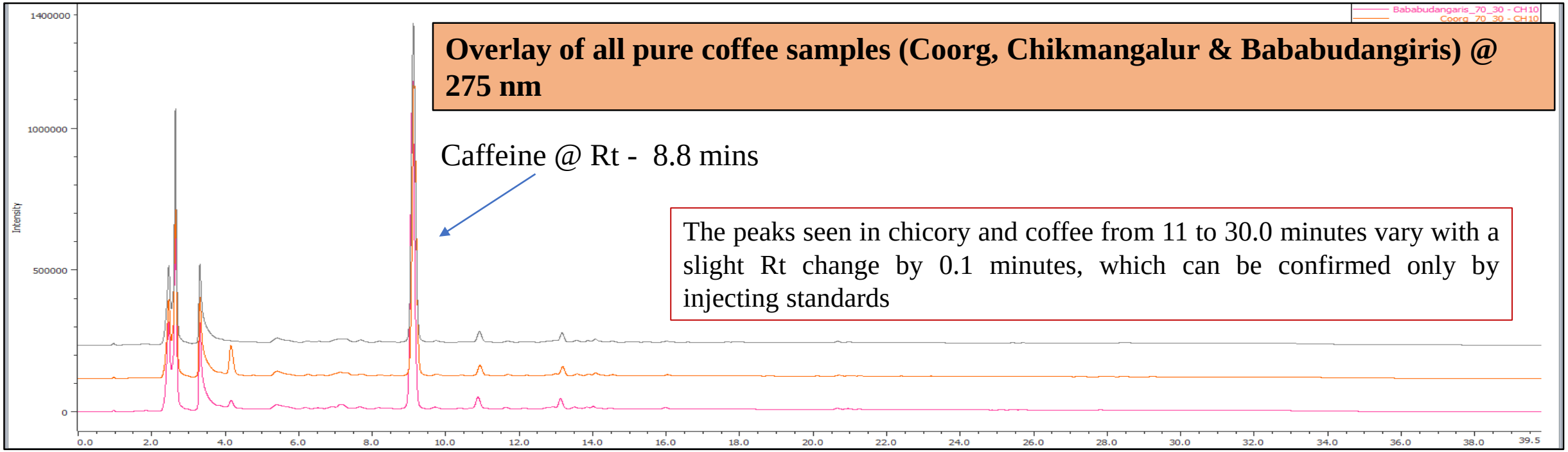
Overlay chromatogram of Processed chicory, commercial sample, and coffee for lactones



- Apart from sesquiterpene lactones a distinct peak at Rt 5.1 min was observed only in the raw chicory and commercial sample, absent in pure coffee (Coorg)


Processed chicory and pure coffee samples @ 275 nm
Unknown peak in processed chicory @ 5.1 mins
Caffeine @ 8.8 mins
Bababudangaris_70_30 - CH10
Coorg_70_30 - CH10
Chikmangaluru_70_30 - CH10
Raw chicory _70_30 - CH10
Intensity
4500000
4000000
2000000
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0
32.0
34.0
36.0
38.0
39.5

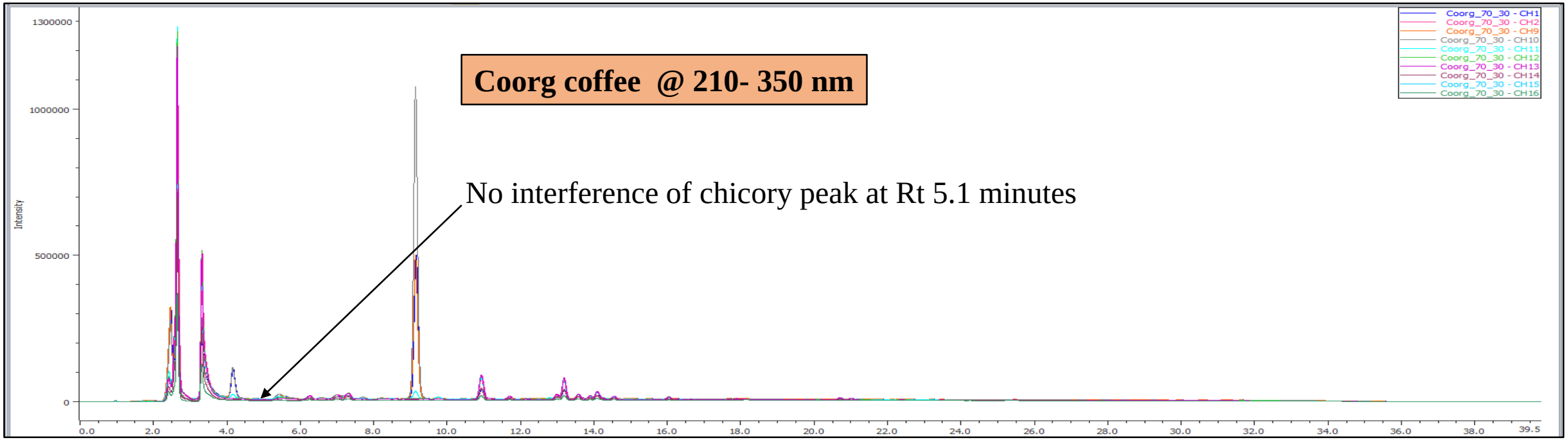

Processed chicory and commercial samples @ 275 nm
Nescafe_70_30 - CH10
Commercial spl_55_45_EtoAc fraction_CHCl3 - CH10
Raw chicory _70_30 - CH10
Intensity
5500000
4000000
2000000
0
Processed chicory
Tata grand 55/45
Nescafe 70/30
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0
32.0
34.0
36.0
38.0
40.0



Overlay of all pure coffee samples (Coorg, Chikmangalur & Bababudangiris) @ 275 nm

Caffeine @ Rt - 8.8 mins

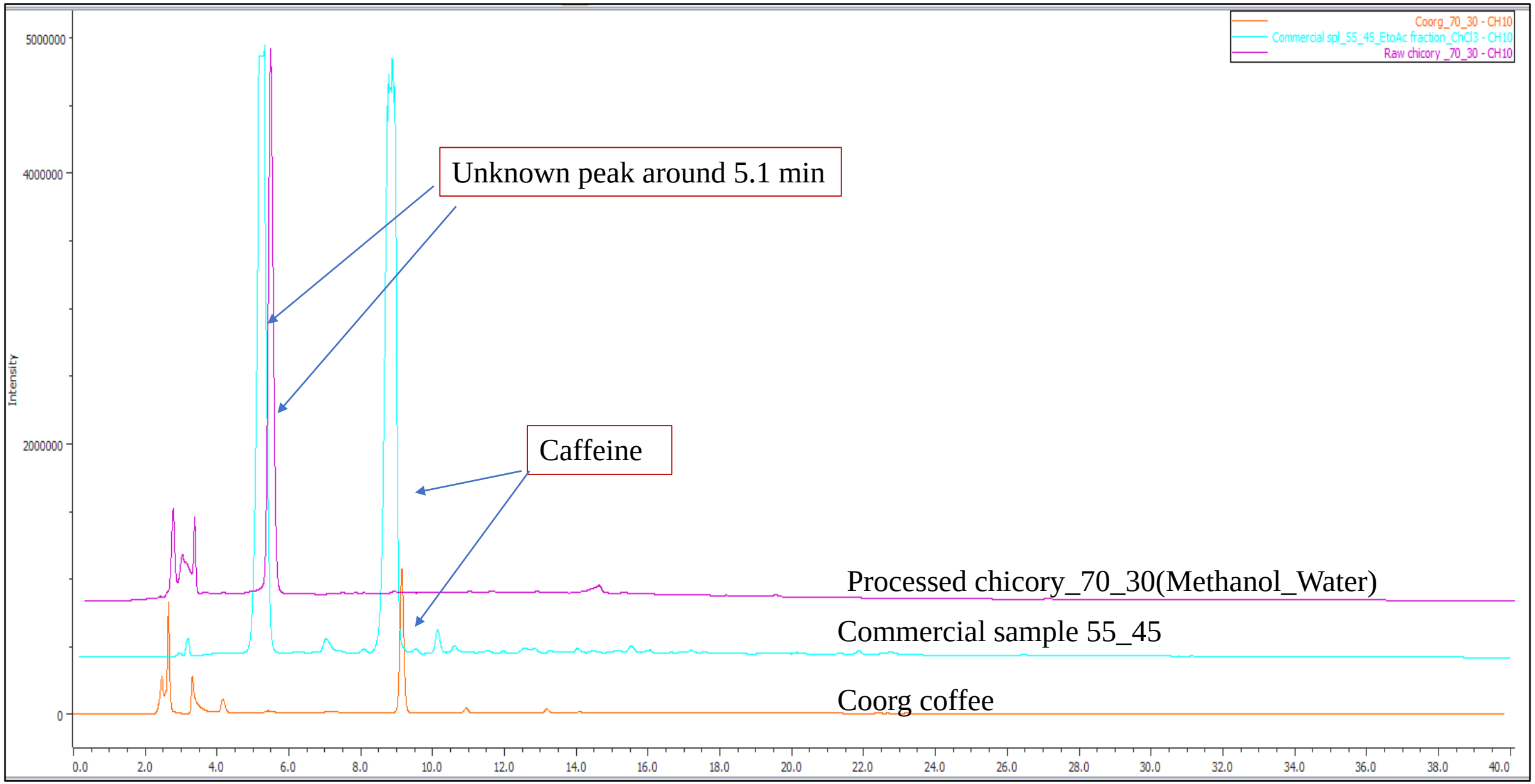
The peaks seen in chicory and coffee from 11 to 30.0 minutes vary with a slight Rt change by 0.1 minutes, which can be confirmed only by injecting standards



Coorg coffee @ 210- 350 nm

No interference of chicory peak at Rt 5.1 minutes

Overlay chromatogram of Processed chicory, commercial sample, and coffee



Outcomes of the study & Future direction

Processed chicory (Ethylacetate fraction)

When subjected to HPLC

Two findings

Similar pattern for **lactones** was observed in comparison with the **reference chromatogram**

Need to be confirmed by injecting the **respective standards**

A peak eluted at **Rt 5.1 minutes** is observed only in **chicory and commercial samples** , absent in all the coffee samples(Coorg, chikmangaluru and babubudangiris)

Particular peak has to be isolated , match the isolated compound with the phytoconstituents of chicory

Identify it using IR, Mass and NMR spectroscopy

If it **unique** it can be considered as a **marker for chicory quantification**