

## HPLC analysis of CGA-7™

### Requirements

Acetonitrile (CH<sub>3</sub>CN), Methanol (CH<sub>3</sub>OH), HPLC water, Formic acid & Trifluoro acetic acid (TFA)

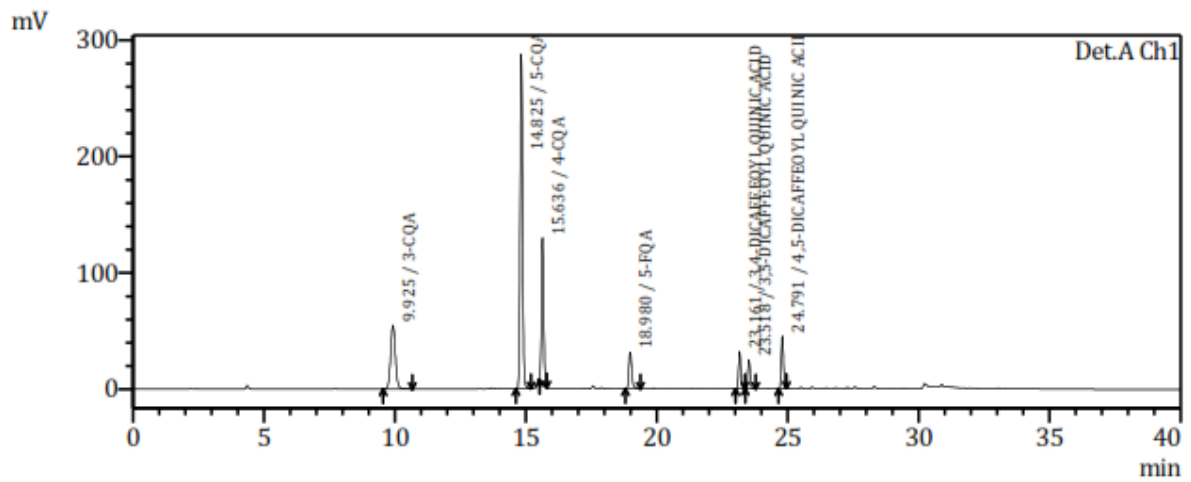
Analytical parameters

HPLC	LC2010cHT Shimadzu
Column	C-18, 5 μm, 100×4.6 mm Phenomenex (Kinetex)
Wavelength	330 nm
Flow rate	1.5 mL/min
Injection volume	3 μL
Column temperature	25 °C
Mobile phase	0.1 % Trifluoroacetic acid in water:CH <sub>3</sub> CN

### Calculation:

$$\% \text{ Total chlorogenic acids} = \frac{\text{Peak area of sample} \times \text{Conc. of standard} \times \text{Purity of standard}}{\text{Peak area of the standard} \times \text{Conc. of the sample}}$$

## HPLC chromatogram of CGA-7



Detector A Ch1 330nm

Peak#	Ret. Time	Name	Area	Height
1	9.925	3-CQA	703798	54897
2	14.825	5-CQA	1872173	287487
3	15.636	4-CQA	728229	124776
4	18.980	5-FQA	245322	31613
5	23.161	3,4-DICAFFEOYL QUINIC ACID	189465	32109
6	24.518	3,5-DICAFFEOYL QUINIC ACID	153037	25011
7	24.791	4,5-DICAFFEOYL QUINIC ACID	262144	45099
Total			4154166	600992

## Normal Diet (Caloric information)

Breakfast		
Food Item	Quantity	Calories (cal)
Idli	100 g	338
Sambhar	200 g	
Upma	20 g	480

Chutney	200 g	
Mix Veg Pulav	130 g	760
Bundhi Raita	60 g	
Chapathi	4 no.	
Channa masala	150 g	
Dosa	3 no.	452
Veg sagu	200 g	
<b>Lunch</b>		
Chapathi	2 no.	230
Vegetable curry	100 g	
Rice	150 g	
Sambhar	200 g	
Rice	150 g	1015
Sambhar	200 g	
Sheera	100 g	
Paratha	3 no.	
Tindli veg	100 g	
Chapathi	2 no.	750
Tomato curry	100 g	
Rice	150 g	
Dal	150 g	
Vegetable biryani	200 g	550
<b>Snacks</b>		
Fruit salad	300 g	270
Sprout masala	150 g	275
Samosa	2 no.	385
Green chutney	20 g	
<b>Dinner</b>		
Chapathi	4 no.	950
Vegetable curry	150 g	
Jeera rice	150 g	
Dal	175 g	
Chapathi	4 no.	1020
Palak curry	150 g	
Ghee rice	150 g	
Vegetable kurma	150 g	
Chapathi	3 no.	830
Channa masala	150 g	
Vegetable pulav	120 g	
Gulab Jamun	1 no.	
Roti	3 no.	825
Brinjal/ladies finger veg	100 g	
Vegetable pulav	150 g	
Bundhi raita	50 g	
Chapathi	3 no.	950
Cabbage veg	100 g	
Rice	150 g	
Sambhar	150 g	

### Non-vegetarian items

1 bowl Chicken Dum Biryani + 1 Bowl Cucumber salad	= 400 cal
Egg mayonnaise sandwich: 2 pieces	= 265 cal
1 bowl rice +chicken curry	= 750 cal
1 mutton Biryani	= 400 cal
Fish meal	= 450 cal
Fish Biryani	= 350 cal

### Sweets

Gulab Jamun 2 Pieces	= 350 cal
Jilebi - 3-4 Pieces	= 490 cal
Kheer - 1 bowl	= 250 cal
Laddu - 2 Pices	= 400 cal

Note: Normal caloric requirement – 2000-2500 cal.

### Study event schedule

	Screening	Visit 1	Visit 2	Visit 3	Visit 4	Follow-up visit
Informed consent process	X					
Medical and surgical history	X					
Prior medications history	X					
Demographic data	X					
Physical examination (including vital signs)	X	X			X	X
DEXA analysis		X			X	
Laboratory assessments*	X	X			X	
Review of I/E criteria	X	X				
Randomization		X				
Dispensing medications		X	X	X		
AE assessment		X	X	X	X	X

Review of concomitant medications		X	X	X	X	X
Review of subject diary (compliance, AEs, Con meds and dietary details)			X	X	X	X
Dispensing of subject diary		X	X	X	X	

\*Laboratory parameters: Serum lipid profile, HbA1c, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, total and direct bilirubin, total blood cell count, urine analysis.

### Sample Size:

The sample size calculation was based on difference of 2 treatments are considered to be medically relevant. Assuming a common SD of 1.5 at the end of treatment, 31 per group would be sufficient to detect a difference of 1.1 in mean difference b/w the 2 treatment with power of 80% and a 0.05. 2-sided level of significance.

### R-Program:

```
power.t.test(n=NULL, delta=1.1, sd=1.5, sig.level=0.05, power=0.80,
             type="two.sample", alternative="two.sided")
```

Two-sample t test power calculation

```
      n = 30.18116
  delta = 1.1
     sd = 1.5
sig.level = 0.05
  power = 0.8
alternative = two.sided
```

NOTE: n is number in \*each\* group

A total of  $N$  number of subjects are required at each Treatment group in the end of the study with all the data being complete for analysis, but a proportion ( $q$ ) are expected to drop out before the study ends. In this case, the following total number of subjects ( $N1$ ) would have to be enrolled to ensure that the final sample size ( $N$ ) in each Treatment group is:

$$N1 = \frac{N}{1 - q} = \frac{31}{(1 - 0.10)} = 35$$

Where  $q$  is the proportion of attrition and is generally 10% in this type of studies.

Note: The proportion of eligible subjects who will refuse to participate (drop out) or provide the inadequate information will be unknown at the beginning of the study. Approximate estimates is often possible using information from similar studies.

