



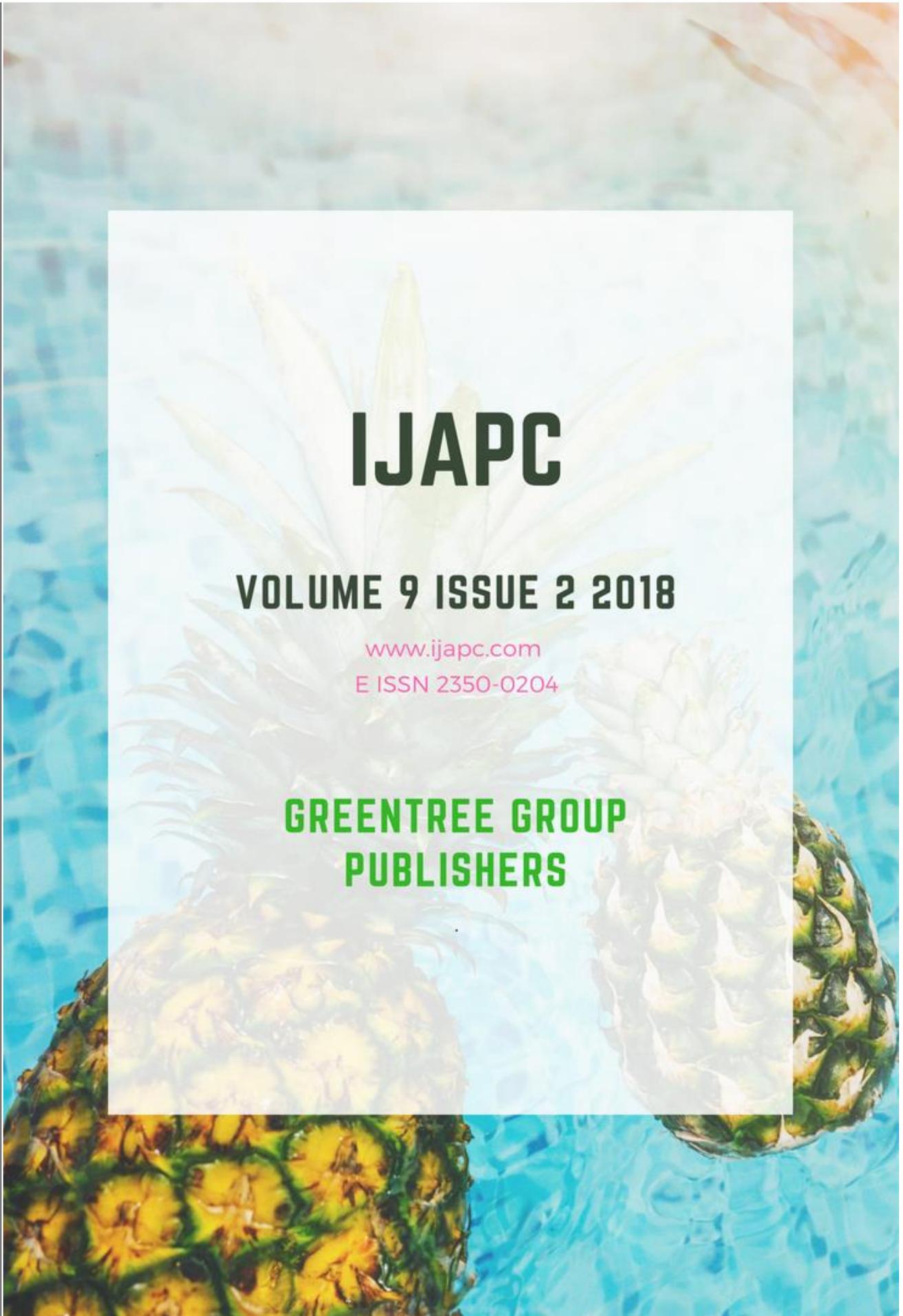
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## Estimation of Calcium Contents and its Bioaccessibility in Different Calcium Tablets

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### ABSTRACT

Calcium is the essential element for normal body growth. Calcium deficiency leads to a decrease in bone density causing osteoporosis. It is observed that many people are unaware of this fact until they meet an accident. It is advisable to have a calcium supplement after age 40. Although calcium tablets are administered, all the calcium may not be bio-accessible. In order to know how much of the calcium actually is absorbed; bio-accessibility studies are important. Taking into consideration the importance of calcium, present work is undertaken to estimate the calcium content and its bio-availability in various calcium tablets by using the flame atomic absorption spectrometer. The studies will be helpful in prescribing the dosage to osteoporotic patients.

### KEYWORDS

*Bio-accessibility, Bio-availability, Calcium, Osteoporosis*



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## INTRODUCTION

Calcium acts in the crucial role in building strong and dense bones in early life and keeping them healthy later in life. Calcium represents the most abundant element in the human body; its vital role in the physiology and biochemistry of organisms and the cells. Calcium readily builds bones and support systems and functions of blood cells<sup>10</sup>. Calcium ions outside the cells are important for cell membranes as well as proper bone formation. For example, contraction and digestive systems, nerve conduction and clotting of blood. Clotting of blood is highly regulated by intra and extra cellular  $\text{Ca}^{2+}$  levels in body. Calcium supplements have also been found to reduce the risk of heart attacks by producing small beneficial changes in both blood pressure and blood cholesterol levels<sup>9</sup>. Calcium plays the important role because  $\text{Ca}^{2+}$  ions form stable complexes with many organic compounds especially proteins. Calcium supplementation is controversial and its bioavailability strongly dependent to a salt's solubility. Vitamin D plays a key role in enhancing phosphorus entry with maintaining an acceptable balance of calcium furthermore within phosphorus in the blood, thereby making it a critical component for good health and strong bones<sup>5</sup>. Elementary levels of Vitamin D can result in insufficient levels

of Calcium, many bone diseases can be traced to problems with the organic matrix in molecular structure<sup>5,8</sup>. Calcium supplements may benefit the serum lipids in women who have passed menopause and in older men. The body does not make enough calcium; the dietary intake or supplementation is necessary. There are several good sources of calcium such as green leafy vegetables, dairy products, nuts, seeds, etc<sup>3</sup>. Calcium supplements are used to prevent and treat calcium deficiencies. Most experts recommend that supplements can be taken with food and that no more than 600 mg should be taken at a time because the amount of calcium absorbed decreases with increasing the amount of calcium in supplement. It is suggested to spread doses throughout the day. The fraction of the total minerals available in the tablets for uptake by the intestinal brush border cell membranes<sup>1</sup>. In medicine, bioavailability obtains a measuring of the extent to that medication reaches systematic circulation<sup>7</sup>. Calcium availability is a measure of the proportion of the total calcium in the tablets that is digested, absorbed and metabolised by normal pathways<sup>11</sup>. In order to estimate calcium bioavailability, several in-vitro methods have been proposed as an alternative to in vivo methods. In vitro method simulation of gastrointestinal



digestion is followed by a determination of amount of calcium soluble; at this level bio-accessibility is measured. In vitro models allows us to manage the conditions of the assay experiment optimally, which may lead to high precision, lower variability, low cost and shorter time needed for results than vivo methods. This study is aimed at estimating the bio accessibility of calcium from tablets using Calcium solubility after gastrointestinal digestion.

## MATERIALS AND METHODS

### Sample Collection:

Different calcium tablets were purchased from medical shops in Pune City and its calcium content was determined by using FES technique<sup>6</sup>.

### Chemicals:

All chemicals used for experiments were of analytical grade and are listed in table 1.

**Table 1** Chemicals used in present work

Name	Make
Nitric Acid	Sd-fine
Perchloric Acid	Sd-fine
Pepsin	SRL
Hydrochloric Acid	Sd-fine
Pancreation	SRL
Bile Salts	SRL
Ammonium bicarbonate	Sd-fine

### Sample Preparation:

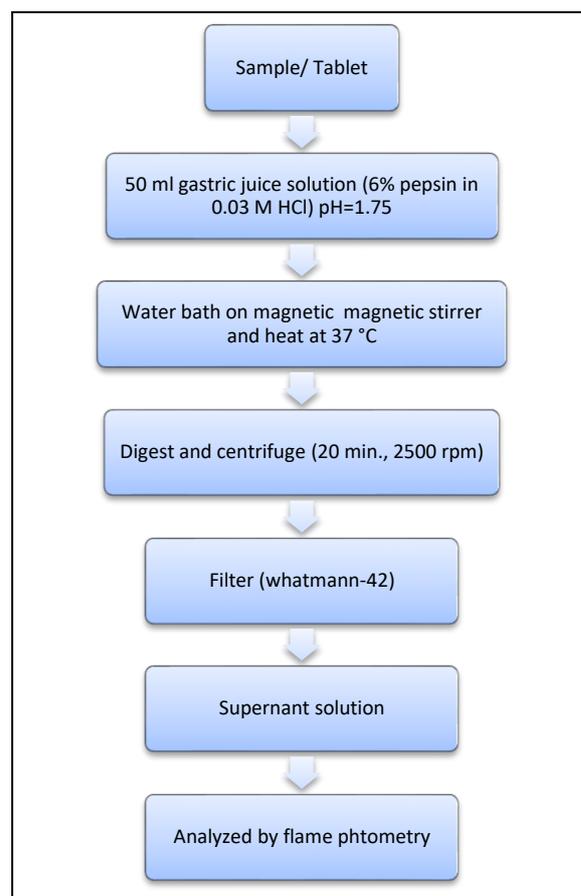
An accurately weighted powder of the calcium tablet is digested with acid mixture [Nitric acid and Perchloric acid (5:1)]. After

digestion, few drops of HCl were added and the solution was filtered and diluted to 25 ml for further analysis.

### Gastric digestion for bio-accessibilities studies:

To 0.5 gm sample in round bottom flask, 50 ml gastric juice solution (6% pepsin in 0.03 M HCl, pH=1.75) was added and shaken vigorously for 2 minutes, it was placed in water bath on magnetic stirrer and heated at 37 °C for 3 hrs, followed by centrifugation for 15 min at 2500 rpm, filtered with whatman (42) and analysed by flame photometry as shown in figure 1.

### Flow Chart of GD:



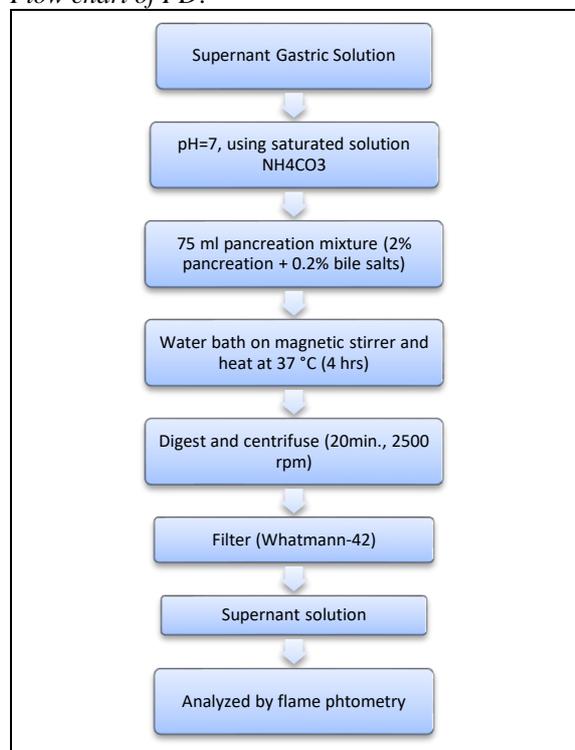
**Fig 1.** Flow chart before Pancreatic digestion for Gastric digestion



### Pancreatic digestion for bioaccessibilities studies:

Completing all steps from gastric digestion (up to centrifuge). The supernatant solution of gastric digestion was used by maintaining the pH =7, thereafter adding  $\text{NH}_4\text{HCO}_3$  saturated solution & 35 ml pancreatic mixture i.e. 2% pancreation + 0.2 % bile salts. At that time it was shaken vigorously for 1 minute. The round bottom flask containing solution was placed in water bath on magnetic stirrer and heated at  $37^\circ\text{C}$  for 4 hrs, followed by cold centrifugation for 20 minutes at 5000 rpm as shown in figure 2. At that point the solution was filtered with Whatman (42) and analysed using flame photometry.

Flow chart of PD:



**Fig 2** Flow chart after Gastric digestion for Pancreatic digestion.

## RESULTS AND DISCUSSION

Present work deals with analysis of calcium tablets for its calcium content and their bio-accessibility. The in-vitro method for estimating availability of essential minerals has gained popularity as it is a simple, reliable, rapid and inexpensive method for predicting the availability of minerals. The in-vitro method can be applied to study both calcium bio-availability and the factors that affect calcium bio-availability in various kinds of foods. Total elemental concentrations of calcium in the commercially available pharmaceutical tablets as well as its bio-accessible concentration are estimated by flame photometry.

### Estimation of calcium using flame photometer

For the determination of total calcium content from samples as shown in the table 2 the calibration curve was prepared by using the standard calcium solutions at different concentrations<sup>6</sup>.

**Table 2** Total calcium content

Tablets	Total weight of tablet	Total calcium amount (mg)
1	1.140	311.448
2	1.379	203.194
3	1.148	313.630
4	0.572	003.907

### Bioaccessibility of Calcium in different Calcium tablets after GD :

Bio-accessibility after gastric digestion as shown in table 3 is calculated using



following equation, % Bio-accessibility =  $([GD] \times 100) / [T]$

[GD] = Element concentration in gastric digestion.

[T] = Total concentration of element.

**Table 3** Bio-accessibility after Gastric Digestion (GD)

Tablet	Total Ca Content in tablet (mg)	Ca Conc. in GD (mg)	Bio-accessibility (%)
1	311.448	12.45	3.99
2	207.194	10.36	5.09
3	313.630	11.76	3.75
4	003.907	0.390	9.99

### Bioaccessibility of Calcium in different Calcium tablets after PD:

Bio accessibility after pancreatic digestion as shown in table 4 is calculated using following equation,

% Bio accessibility =  $([PD] \times 100) / [T]$

[PD] = Element concentration in pancreatic digestion

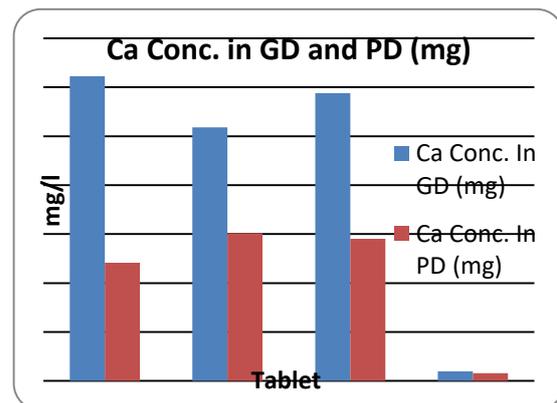
[T] = Total concentration of element in sample.

**Table 4.** Bio-accessibility after pancreatic digestion.

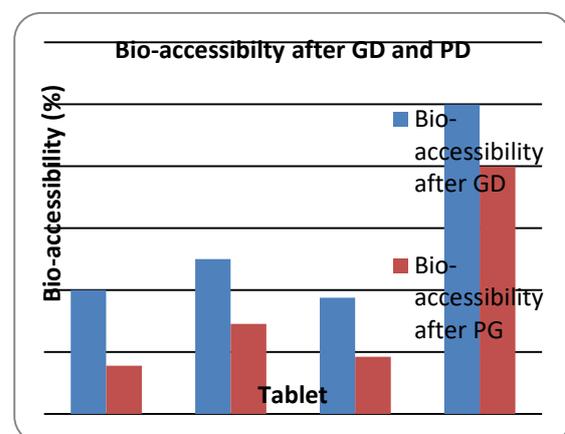
Tablets	Total Ca Content in tablet (mg)	Ca conc. in PD (mg)	Bio-accessibility (%)
1	311.448	4.827	1.55
2	207.194	6.028	2.90
3	313.630	5.802	1.84
4	003.907	0.312	7.99

As concerting from table 2 ,the tablet-I and III are good sources of calcium. However, if you can examine the bioaccessibility it is found to be in range 3.75-10% as shown in figure 4. Thus even though calcium tablets are consumed by the patient, all the calcium

is unabsorbed by the human body but only fraction of it is getting absorbed. Tablet-II and IV are having relatively good bioaccessibility as compare to I and III as shown in figure 4. This is principally because of the fact that these tablets contain Vitamin D which helps in calcium absorption. More further examination of four tablets reveals that bioaccessibility after gastric digestion is more than that of pancreatic digestion as shown in table 3 and table 4. This is the consistent with the fact that Calcium absorption is more in acidic medium.



**Fig 3** Ca Concentration in Gastric digestion (GD) and Pancreatic Digestion (PD).



**Fig 4** Bio-accessibility after Gastric digestion (GD) and Pancreatic digestion (PD)



## CONCLUSION

Flame photometry was applied to determine the bio-accessibility of calcium elements from pharmaceutical tablets using in vitro gastric and pancreatic digestion methods. Comparatively, bio-accessibility during gastric digestions was higher than pancreatic digestion indicating higher absorption of calcium in acidic medium. The results obtained clearly demonstrate that all tested tablets have relatively good amount of Calcium but relatively low bio-accessibility.



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