Pharmacology and pharmacodynamics of hCG solutions to be administered by the oral sublingual approach.

The influence of different excipients and storage procedures.
• **Authors:**

1. Dr. Sergio Ariel Vaney  
   PhD-Pharmacist

2. Daniel Oscar Belluscio  
   MD

The oral hCG Research Center  
Guido1953- 1119 Buenos Aires  
Argentina  
Email: hcgobesity@fibertel.com.ar

© Dr. Sergio Ariel Vaney and Dr. Daniel Oscar Belluscio. All rights reserved (2011)
Introduction

• Currently Human Chorionic Gonadotropin (hCG), both the version obtained from urine of pregnant women or from recombinant DNA, can be obtained in the market in the form of liquid vials or lyophilized powder for injectable administration.

• Our current research provided us an insight to advance in the development for a new formulation, or pharmaceutical presentation for hCG, to be administered by the oral-sublingual route.

• Our objective is to develop a novel formulation or pharmaceutical liquid form of hCG with sufficient stability to grant a therapeutically effective presentation of hCG apt to be used by the oral/sublingual approach.
Theoretical Aspects:
- Analysis and study of pharmacotechnical aspects to assess the ideal conditions for obtaining stability in the proposed Oral hCG formulation.
hCG stability study: analytical method and results.
Human Chorionic Gonadotropin in liquid pharmaceutical form

- **Objective:**
  - The following study aims to establish the conditions that will provide a stable pharmaceutical preparation of a liquid formulation of hCG for oral administration.

- **Summary:**
  - We propose an accelerated stability study in order to evaluate variables that could affect the chemical stability of hCG in a liquid solution.
Study Design: Objectives

- Gather information as regards the stability of hCG in a liquid environment.
- Need to establish variables or parameters evaluating the biological, physical and chemical properties of hCG under different conditions.
- These considerations will determine the analytical methods that will allow us to formulate those stability environments to quantifiable parameters.
- Perform preliminary studies to assess four study parameters or variables that in our opinion can affect the chemical stability of hCG in a liquid solution.
HCG Stability Study: analytical method and results

Defined Study Parameters:

1. PH.

2. Ionic Force (influence of electrolytes.)


4. Temperature.
HCG Stability Study: analytical method and results

Materials

- 5000 IU Human Chorionic Gonadotropin– lyophilized (GONACOR 5000 – Massone Institute)
- Phosphoric Acid 85% (Carlo Erba)
- Sodium Hydroxide (Merck – Analytical Grade)
- Sodium Chloride (Merck – Analytical Grade)
- Injectable quality distilled water (Roux Ocefa)
Method

- The samples under study were submitted to accelerated stability conditions: they are maintained at temperatures from 40ºC to 50ºC during approximately 12 weeks.
- We will define as quantifiable parameter the purity (hCG concentration) according to time.
- The quantification of the concentration parameter will be established through an analytical method called HPSEC or High Performance Size-Exclusion (Molecular exclusion chromatography.)
- This chromatographic method allows the separation of substances according to their molecular weight and is used for the separation of proteins and substances with high molecular weight.
**HCG Stability Study: analytical method and results**

**Standard working conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase A</td>
<td>0.1M phosphate pH 6.7 + 0.1M Sodium sulfate</td>
</tr>
<tr>
<td>Isocratic conditions</td>
<td>100% phase A.</td>
</tr>
<tr>
<td>Column</td>
<td>TSK G 2000 SWXL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.5 ml/min</td>
</tr>
<tr>
<td>UV Detector</td>
<td>214 nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>40 microlite (5000 IU)</td>
</tr>
</tbody>
</table>
Sample Preparation

- All samples subject to the following study were elaborated according to operative conditions established in the following order:

  1. The hCG was diluted in the adequate solvent (injectable quality distilled water) and homogenized.

  2. The pH of the obtained solution was adjusted with Phosphoric Acid at 85% and Sodium Hydroxide 1M solution to reach pH7.

  3. The obtained solution was filtered through sterile syringe filters of 0.22 microns (Minisart 16534 K – cellulose acetate) to guarantee solution sterility.

  4. The obtained solution was bottled in 10 cc. vials and the vials were tapped with rubber tops and aluminum security precincts.

  5. All described operations were performed under laminar flux conditions.
Graphs results to evaluate the chemical stability of hCG according to the four established parameters or variables.
To evaluate the effect of pH on the chemical stability of hCG in liquid solution, three samples were prepared. Their composition can be observed in Table 1:

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
</table>
| **Fosf 6** | HCG 5000 IU  
Phosphoric acid 85% 0.98mg  
Sodium Hydroxide solution 1M q.s to PH 6  
Water injection q.s to 1 ml |
| **Fosf 7** | HCG 5000 IU  
Phosphoric acid 85% 0.98mg  
Sodium Hydroxide solution 1M q.s to PH 7  
Water injection q.s to 1 ml |
| **Fosf 8** | HCG 5000 IU  
Phosphoric acid 85% 0.98mg  
Sodium Hydroxide solution 1M q.s to PH 8  
Water injection q.s to 1 ml |
Here in Graphs 1 and 2 we display the stability curves obtained for samples under experimental conditions:

**PH effect on the HCG Purity (HCG %)**
Temperature 50°C = 154°F

Graph 1
PH effect on the HCG Purity (HCG %)

Temperature 40°C = 123.2°F

Graph 2
HCG Stability Study: analytical method and results

- Influence of pH on Purity (% hCG)

### Temperature 50°C = 154°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 week</th>
<th>Time 1 week</th>
<th>Time 3 week</th>
<th>Time 5 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 6</td>
<td>100</td>
<td>94.1</td>
<td>90.76</td>
<td>81</td>
</tr>
<tr>
<td>Fosf 7</td>
<td>100</td>
<td>96.09</td>
<td>93.12</td>
<td>86.93</td>
</tr>
<tr>
<td>Fosf 8</td>
<td>100</td>
<td>94.21</td>
<td>82.50</td>
<td>74.96</td>
</tr>
</tbody>
</table>

### Temperature 40°C = 123.2°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 week</th>
<th>Time 3 week</th>
<th>Time 5 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 6</td>
<td>100</td>
<td>97.5</td>
<td>93</td>
</tr>
<tr>
<td>Fosf 7</td>
<td>100</td>
<td>96.72</td>
<td>93.74</td>
</tr>
<tr>
<td>Fosf 8</td>
<td>100</td>
<td>96.77</td>
<td>93.55</td>
</tr>
</tbody>
</table>
2 – Ionic Force (influence of electrolytes)

- To assess the influence of electrolytes on the chemical stability of hCG in liquid solution, 2 samples were prepared, their composition can be observed in Table 2:

Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / 4,4 mg/ml (150 mOsm)</td>
<td>HCG 5000 IU / ml</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride 4,4 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Phosphoric acid 85% 0.98mg</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide solution 1M q.s to PH 7</td>
</tr>
<tr>
<td></td>
<td>Water injection q.s to 1 ml</td>
</tr>
<tr>
<td>Fosf 7 / 8,8 mg/ml (300 mOsm)</td>
<td>HCG 5000 IU / ml</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride 8,8 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Phosphoric acid 85% 0.98mg</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide solution 1M q.s to PH 7</td>
</tr>
<tr>
<td></td>
<td>Water injection q.s to 1ml</td>
</tr>
</tbody>
</table>
In Graph 3 and Graph 4 we can observe the stability curves obtained for both samples.

The samples have been evaluated at temperatures of 25ºC and 4ºC, additionally to the proposed experimental conditions.
Electrolites effect on the Purity (%HCG)
Temperature 40°C = 123,2°F

Graph 4
HCG Stability Study: analytical method and results

- Influence of Electrolytes (Ionic Force) on Purity (%HCG)

<table>
<thead>
<tr>
<th>Temperature 50°C = 154°F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
</tr>
<tr>
<td>Fosf 7 / 4,4mg/ml</td>
</tr>
<tr>
<td>Fosf 7 / 8,8mg/ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature 40°C = 123,2°F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
</tr>
<tr>
<td>Fosf 7 / 4,4mg/ml</td>
</tr>
<tr>
<td>Fosf 7 / 8,8mg/ml</td>
</tr>
</tbody>
</table>
## HCG Stability Study: analytical method and results

- **Influence of Electrolytes (Ionic Force) on Purity (%HCG) (2)**

### Temperature 25°C = 77°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 week</th>
<th>Time 3 week</th>
<th>Time 6 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / 4,4mg/ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fosf 7 / 8,8mg/ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### Temperature 4°C = 12.32°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 week</th>
<th>Time 2 week</th>
<th>Time 4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / 4,4mg/ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fosf 7 / 8,8mg/ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Electrolites effect on the Purity (%HCG)
Temperature 25°C = 77°F and 4°C = 12.32°F

Graph 5
Conclusion

- In proposed experimental conditions (temperatures of 50ºC and 40ºC) we observe that an increase in concentration of electrolytes negatively affects purity (%hCG) of Human Chorionic Gonadotropin in liquid solution.

- No changes are observed at temperatures between 25ºC and 4ºC.
Comparative Study- Effect of different Electrolytes: Silver

- In order to qualitatively evaluate the influence of electrolytes on the chemical stability of hCG in liquid solutions, two samples were analyzed by ultraviolet spectrophotometry. Their composition is observed in Table 3:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / Nacl</td>
<td>HCG 5000 IU / 10ml Sodium chloride 8,8 mg/ml Phosphoric acid 85% 0.98mg Sodium hydroxide solution 1M q.s to PH 7 Water injection q.s to 10 ml</td>
</tr>
<tr>
<td>Fosf 7 / AgNO₃</td>
<td>HCG 5000 IU / ml Silver nitrate 5 mg/ml Phosphoric acid 85% 0.98mg Sodium hydroxide solution 1M q.s to PH 7 Water injection q.s to 10 ml</td>
</tr>
</tbody>
</table>
HCG Stability Study: analytical method and results

Working Conditions

- The samples under study were analyzed by a Beckman 25 spectrophotometer, scanning between 300nm–220nm, using quartz buckets of 10 mm of width and maintaining temperature between 22ºC–25ºC in a thermostatic bath.
- The obtained specters of both samples are displayed in Graph 5:

![Graphic 5](image-url)

**Electrolites effect**
UV-Spectrophotometer
Temperature 25ºC = 77ºF

Absorbance vs. Wavelength (nm)

- HCG Fosf 7 / 8.8mg/ml
- HCG Fosf 7 / AgNO3 5mg/ml
- HCG Fosf 7 / water
Conclusions

- The spectrophotometric comparative study of both samples evidences that the use of sodium chloride as electrolyte favors the chemical stability of hCG in liquid solution.

- By contrast, the use of silver salts in liquid solution degradates the hCG molecule.
3 – Influence of Excipients

- To compare the effect of excipients on the chemical stability of hCG in liquid solution, two samples were prepared. Their composition can be observed in Table 4.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / mannitol</td>
<td>HCG 5000 IU / ml</td>
</tr>
<tr>
<td></td>
<td>Mannitol 54 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Phosphoric acid 85% 0.98mg/ml</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide solution 1M q.s to PH 7</td>
</tr>
<tr>
<td></td>
<td>Water injection q.s to 1 ml</td>
</tr>
<tr>
<td>Fosf 7 / Sucrose</td>
<td>HCG 5000 IU / ml</td>
</tr>
<tr>
<td></td>
<td>Sucrose 102 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Phosphoric acid 85% 0.98mg/ml</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide solution 1M q.s to PH 7</td>
</tr>
<tr>
<td></td>
<td>Water injection q.s to 1 ml</td>
</tr>
</tbody>
</table>
• We can observe the stability data obtained for both samples in Graph 7 and Graph 8.
• Samples were also subjected to temperatures of 25°C and 4°C.

Excipients effect on the Purity (%HCG)
Temperature 50°C = 154°F

- Fosf 7 / mannitol
- Fosf 7 / Sucrose

Graph 7
HCG Stability Study: analytical method and results

Time (weeks)

Graph 7
HCG Stability Study: analytical method and results

Excipients effect on the Purity (%HCG)
Temperature 40ºC = 123,2ºF

Graph 8
HCG Stability Study: analytical method and results

- Influence of excipients on Purity (%hCG)

<table>
<thead>
<tr>
<th></th>
<th>Temperature 50ºC = 154ºF</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td></td>
<td>Time 0 (weeks)</td>
<td>Time 1 (weeks)</td>
<td>Time 2 (weeks)</td>
</tr>
<tr>
<td>Fosf 7 / mannitol</td>
<td>100</td>
<td>94</td>
<td>91.7</td>
<td>83.5</td>
</tr>
<tr>
<td>Fosf 7 / Sucrose</td>
<td>100</td>
<td>94.1</td>
<td>90.3</td>
<td>83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Temperature 40ºC = 123,2ºF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td></td>
<td>Time 0 (weeks)</td>
<td>Time 2 (weeks)</td>
</tr>
<tr>
<td>Fosf 7 / mannitol</td>
<td>100</td>
<td>97.9</td>
<td>97.1</td>
</tr>
<tr>
<td>Fosf 7 / Sucrose</td>
<td>100</td>
<td>98</td>
<td>95.5</td>
</tr>
</tbody>
</table>
HCG Stability Study: analytical method and results

- Influence of excipients on Purity (%hCG) (2)

## Temperature 25°C = 77°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 (weeks)</th>
<th>Time 3 (weeks)</th>
<th>Time 6 (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / mannitol</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fosf 7 / Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

## Temperature 4°C = 12,32°F

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time 0 (weeks)</th>
<th>Time 2 (weeks)</th>
<th>Time 4 (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosf 7 / mannitol</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fosf 7 / Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
HCG Stability Study: analytical method and results

Comparative Study – Excipients

- To qualitatively evaluate the influence of excipients on the chemical stability of hCG in liquid solutions, two samples were analyzed by ultraviolet spectrophotometry (400nm–200nm). Their composition is observed in Table 5:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG Fosf 7 / Water</td>
<td>HCG 5000 IU / 10ml&lt;br&gt;Phosphoric acid 85% 0.98mg&lt;br&gt;Sodium hydroxyde solution 1M q.s to PH 7&lt;br&gt;Water injection q.s to 10 ml</td>
</tr>
<tr>
<td>HCG Fosf 7 / Ethylc alcohol</td>
<td>HCG 5000 IU / ml Ethylc alcohol 5% v/v Phosphoric acid 85% 0.98mg&lt;br&gt;Sodium hydroxyde solution 1M q.s to PH 7&lt;br&gt;Water injection q.s to 10ml</td>
</tr>
</tbody>
</table>
HCG Stability Study: analytical method and results

Working Conditions:
- The samples under study were analyzed by a Beckman 25 spectrophotometer, scanning between 300nm–220nm, using quartz buckets of 10 mm of width and maintaining temperature between 22°C–25°C in a thermostatic bath.
- The obtained specters of both samples are observed in Graph 9:

**Excipients effect**
UV-Spectrophotometer
Temperature 25°C = 77°F

Graph 9
Conclusion:

- The spectrophotometric comparative study of both samples evidences that the use of ethyl alcohol as excipient has a negative effect of the chemical stability of hCG in liquid solution.
HCG Stability Study: analytical method and results

4 – Temperature

- To assess the effect of temperature on the chemical stability of hCG in liquid solution, two groups of samples were prepared as observed in Table 6.
- Both groups were submitted to temperatures of 25ºC, 55ºC, 65ºC, 80ºC and then analyzed by HPLC or High Performance Liquid Chromatograph.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
</table>
| A, C, E, G. | HCG 15000 IU / ml  
Buffer Phosphate 0.01M  
Sodium hydroxide solution 1M q.s to PH 7  
Water injection q.s to 1 ml |
| B, D, F, H. | HCG 15000 IU / ml  
Sodium chloride 0.15M  
Sodium hydroxide solution 1M q.s to PH 7  
Water injection q.s to 1 ml |
# Working Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution</td>
<td>Solution 0.1M potassium phosphate, PH 7 + solution 0.15M sodium chloride</td>
</tr>
<tr>
<td>Column</td>
<td>TSK – G 3000 SW</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>UV Detector</td>
<td>280 nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 microliter</td>
</tr>
</tbody>
</table>
Graph 10 shows chromatographic results obtained for both sample groups.

In both cases we can observe that temperatures above 25°C initiate the process of pharmacologic modifications of the hCG molecule.

These changes are evidenced as signs or peaks that appear in different elusion times (14 and 17 minutes) and correspond to the dissociation of the hCG molecule into its alpha and beta subunits.
According to this we can propose the following expression:

\[ K_d \]

\[ \text{HCG} \leftrightarrow \alpha + \beta \]

\((K_d = \text{dissociation constant})\)

This constant can be calculated by studying the kinetic reaction of hCG at different temperatures.

In Graph 11 and 12 we visualize the curves obtained through fluorescence (analytical method that allows us to measure light emissions- fluorescence) produced by certain substances in solution.
**HCG Stability Study: analytical method and results**

- The capacity of substances in solution to produce fluorescence will depend on: concentration, PH, temperature, presence of electrolytes, presence of other fluorescent substances.

- The sample under study (hCG) is treated with ANS (1,8 anilinonaphthalene sulphonate) fluorescent substance, and its fluorescence is measured given time at a determined temperature. Composition of samples is shown in Table 7:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (black dots)</td>
<td>HCG 1500 IU / ml&lt;br&gt;Buffer Phosphate 0.01M&lt;br&gt;Sodium hydroxide solution 1M qs to PH 7&lt;br&gt;Sodium chloride 0.15M&lt;br&gt;Water injection q.s to 1 ml</td>
</tr>
<tr>
<td>B (white dots)</td>
<td>HCG 15000 IU / ml&lt;br&gt;Buffer Phosphate 0.01M&lt;br&gt;Sodium hydroxide solution 1M q.s to PH 7&lt;br&gt;Water injection q.s to 1 ml</td>
</tr>
</tbody>
</table>
HCG Stability Study: analytical method and results

- On the curves obtained we can see that the dissociation reaction corresponds to a kinetic curve of 1st order represented in the following equations:

\[
\begin{align*}
\log C &= \log C_0 - kd \cdot t / 2.303 \\
t_{1/2} &= 0.693 / kd \\
t_{90} &= 0.105 / kd
\end{align*}
\]

- Applying the Arrhenius equation to results obtained in Graph 9, we obtain Graph 10:

\[
\begin{align*}
\log K_2 / K_1 &= -\frac{E_a}{2 \cdot 303 R} (1/T_2 - 1/T_1) \\
K_2 &= \text{dissociation constant at } T_2 \\
K_1 &= \text{dissociation constant at } T_1
\end{align*}
\]
Observations

- According to the straight line obtained in Graph 12 it is possible to infer (by extrapolation) that the value of the dissociation constant at a temperature of 37ºC has a value of 0.003 (minutes⁻¹).

- Our conclusion is that in physiological conditions of PH and Temperature, the half life of hCG is of approximately 40 hours.
Conclusions

✓ 1) PH
• hCG resulted more stable in a liquid solution at a pH of 7, over pH 6 and pH 8.

✓ 2) Temperature
• The hCG molecule is thermolabile. Thus, at a temperature above 25°C its alpha and beta units are dissociated.

✓ 3) Electrolytes
• The presence of electrolytes or salts such as Sodium Chloride synergize the dissociation of the hCG molecule.

✓ 4) Half-life
• In physiological conditions of PH and temperature, the half life of hCG is of approximately 40 hours.
Conclusions (II):

5) Ionic Force (influence of electrolytes)

- The chemical stability of hCG is negatively affected by the presence of electrolytes at temperatures of 50°C and 40°C. However, at temperatures between 4°C and 25°C the chemical stability depends on the electrolyte present in the solution.

- The comparative spectrophotometric study between two electrolytes (sodium chloride and silver nitrate) demonstrated that the use of sodium chloride favors the chemical stability of hCG in liquid solutions.
Conclusions (III):

6) **Excipients**
- The chemical stability of hCG is not modified by the actions of reduction sugars or poly alcohols at temperatures between 4ºC and 25ºC.

7) **Alcohol- Silver**
- The use of ethyl alcohol and/or silver as excipients have a negative effect of the chemical stability of hCG in liquid solution.