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**Research Article**

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# Purification of hCG Hormone with Enhanced Biopotency from Urine of Pregnant Women

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## ABSTRACT

*A higher biopotency of 26053.93 IU/mg was obtained from the urine of pregnant women using different purification methods. The bioassay of hCG activity was tested on rats based on rat seminal vesicles weight gain. Pregnant women urine was extracted and purified with ion exchange chromatography and were purified from 50 IU to 1500 IU with protein content from 243 mg to 8.12 mg. As a consequence of these results, 4.23 mg of protein content was obtained with a biological activity of 3000 IU/mg. To attain increased purity of HCG, the product was subjected and allowed to pass through the affinity column chromatography. This led to the formation of the highly purified HCG with >26000 IU/mg of biological activity.*

**Keywords:** hCG extract; Pregnant women; Column chromatography

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## INTRODUCTION

The name human chorionoic gonadotropin (hCG) was originated from two different terms. The word “Chorion” is taken from the Latin term referring to “chordate” inferring “after birth”, while the term gonadotropin was retrieved from the “gonad tropic hormone” molecule that acts on ovaries promoting the steroid production. hCG is a glycoprotein, composed of two α and β subunits with 92 amino acid residues in α subunit and 121 aminoacids in β subunit. They are bound by non covalent hydrophobic and ionic interactions. The subunits contain high amounts of mannose with a molecular weight of hCG around 36,000. In this the sugar moiety forms 25-41% of the molecular weight of the total molecular weight of hCG. hCG family represents one such biomarker [1] and hence used in the detection and monitoring pregnancy[2] and also as a biomarker for trophobalstic diseases, cancer prognosis and down syndrome [3-5]. The hCG is produced by the syncytiotrophoblast cells of the fertilized ova/eggs which can be detected only after implantation [6]. In the first trimester, hCG interacts with its receptor LHCG and thus facilities the maintenance of the corpus luteum, allowing it to secrete the hormone progesterone [7], with levels increasing of about 60% for every two days [8]. thereafter tends to plateau or ever drop off [9]. hCG is to make sure that the fetus is provided with the essential nutrients required for its proper growth by effectively utilizing the fat stored in the body of the pregnant women [9]. In continuation of our earlier works [10-12], a higher biopotency of about 26000 IU/mg was obtained in the present study using different purification strategies which are discussed in this communication.

## EXPERIMENTAL SECTION

The process of extraction was similar to that discussed in our earlier work [10-12]. The bioassay of hCG activity was tested on rats based on rat seminal vesicles weight gain. The research project animal experimentation was taken approval from Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) by the Institutional Ethical Committee(IAEC) Form B Approval No: CPCSEA/IAEC/JLS/001/01/14/003 in Jeeva Life Sciences (CPCSEA Reg No:1757/PO/RcBiBt/S/14/CPCSEA), situated in Uppal Hyderabad.

19-28 days SD male old rats were obtained from National Institute of Nutrition (NIN).The animals were housed in laminar air-flow cabinets under pathogen – free conditions with a 12 h light/12 h dark schedule, and were fed autoclaved standard chow and water ad libitium. They were randomized and are injected with a given amount of hCG, for 4 days as per British Pharmacopeia. Four days after start the treatment, on the fifth day, the rats are sacrificed under anesthesia and increased seminal vesicles and of the prostate gland were collected and then weighed. The data retrieved is processed to understand the hCG activity. The potency of chorionic gonadotropin is estimated by comparing under given conditions its effect of increasing the mass of the seminal vesicles (or the prostate glands) of immature rats with the same effect of the International Standard of chorionic gonadotropin or of a reference preparation calibrated in international Units. Used immature SD male rats of the same strain,19 to 28 days old, differing in age by not more than 3 days and having body masses such that the difference between the heaviest and the lightest rat was not more than 10 grams (Figure 1).

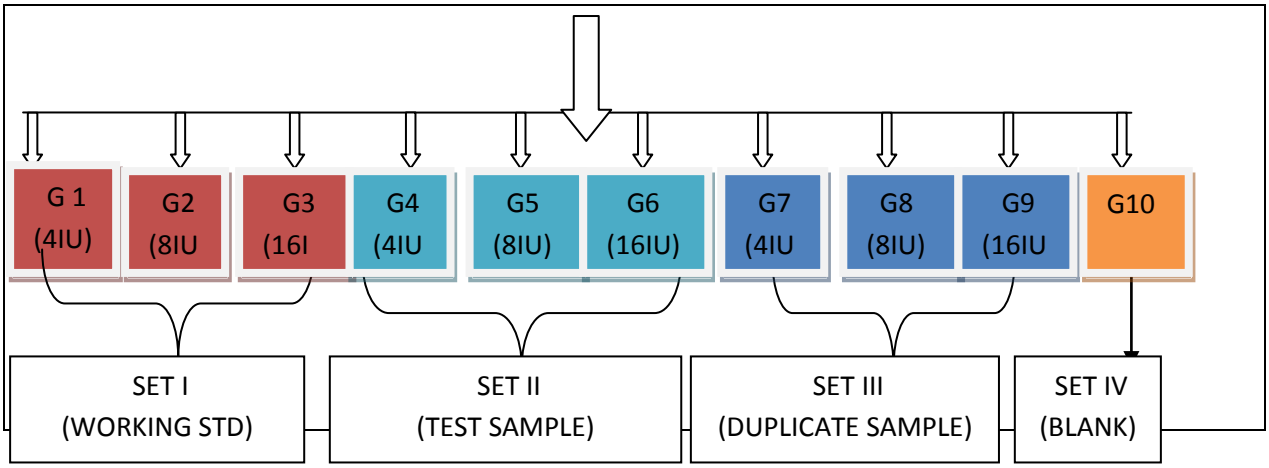


**Figure 1: Showing the schematic representation of four sets where the G1,G2,G3 is SET I (WORKING STD) G4,G5,G6 is SET II (TEST SAMPLE) G7,G8,G9 SET III (DUPLICATE SAMPLE) with 4 IU, 8 IU and 16 IU concentration doses where G10 stands for Blank SET IV**

Assigned the rats at random to 10 equal groups of a 6 animals assigned one litter mate from each set to that the each group are marked according to litter. Chosen 3 doses of the standard reference preparation and 3 doses of the preparation to be examined such that the smallest dose 4 IU is sufficient to produce a positive response in some of the rats and the largest dose does not produce the maximal response in all the rats. Used doses in geometric progression and as an initial approximation total doses of 4 IU, 8 IU and 16IU (although the doses will depend on the sensitivity of the animals used, which may vary widely). Dissolved separately the total quantities of the preparation to be examined and of the reference. Prepared correspondingly to the daily doses to be used in sufficiently phosphate-albumin buffered saline pH 7.2 R such that the daily dose was administered in a volume of about 0.5 ml. Added a suitable antimicrobial preservative such as 4 g/l of phenol or 0.02 g/l of thiomersal. Stored the solutions at 5 ± 3°C. Injected subcutaneously into each rat the daily dose allocated to its group, on 4 consecutive days at the same time each day. On the fifth day, about 24 h after the last injection, euthanized the rats and removed the seminal vesicles. Removed any extraneous fluid and tissue and weighed the vesicles immediately. Calculated the results by the usual statistical methods, using the mass of the vesicles as the response (The precision of the assay may be improved by a suitable correction of the organ mass with reference to the body mass of the animal from which it was taken; an analysis of covariance may be used).

## RESULTS AND DISCUSSION

The estimated potency was not less than 80 percent and not more than 156 percent of the stated potency. The confidence limits (P=0.95) of the estimated potency are not less than 64 percent and not more than 156 percent of the stated potency. Pregnant women urine was extracted and purified with ion exchange chromatography and were purified from 50 IU to 1500 IU with protein content from 243 mg to 8.12 mg. As a consequence of these results, 4.23 mg of protein content was obtained with a biological activity of 3000 IU/mg. To attain increased purity of HCG, the product was subjected and allowed to pass through the affinity column chromatography. This led to the different formation of the highly purified HCG with >26000 IU/mg of biological activity. The measurement of the activity at every step was performed in the rats with 4 set experimental setup (Table 1).

Set-1 was the working standard with dilution details, 2044.11 IU/Vial dissolved in 50 ml (40.88 IU/ml) --->10.00 ml--->made upto 25.55 ml (16.00 IU/ml) --> 10 ml--->made up to 20 ml, (8.00 IU/ml) 10 ml--> made upto 20 ml (4.00 IU/ml). Set-2 Dilution details for sample single assay were same as in the previous experiments but had different potency 26000IU × 10mg dissolved in 200 ml (1300.00 IU/ml) →10.00 ml→ made upto 100 ml (130.00 IU/ml) 10 ml→ 81.25 ml (16.00 IU/ml), 10 ml→ made upto 20 ml (8.00 IU/ml) 10 ml→ made upto 20 ml (4.00 IU/ml). The rest of the parameters, however, remained the same. Set-3 was the sample duplicate assay in which 26000 IU × 10 mg dissolved in 200 ml (1300.00 IU/ml) →10.00 ml→ made upto 100 ml (130.00 IU/ml) 10 ml→ 81.25 ml (16.00 IU/ml) 10 ml→ made upto 20 ml (8.00 IU/ml) 10 ml→ made upto 20 ml (4.00 IU/ml) and set-4 was blank assay with phosphate buffer. It can be seen that the biological activity of the hCG gradually increased as protein purity increased. The results of the potency are shown in Table 2 where the average potency of hCG was shown to be around 26053.93 IU/mg.

**Table 1: Bioassay results - 26000 IU/mg purification protocols**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Product Name:** | | | **HCG -26000 IU/mg (B.P.)** | | | | | | | | | | |
| **Batch No:** | | | **PHD 006** | | **Date of analysis:** | | | 13.10.2015 | | | | | |
| **Reg. No:** | | | **TLR/11/01001/14** | | **Date of completion:** | | | 17.10.2015 | | | | | |
| **Label claim:** | | | **26000 IU /mg** | | **Date of report:** | | | 21.10.2015 | | | | | |
| **Date of Mfg:** | | | **Sep' 2015** | | | | | | | | | | |
| **Protocol No.** | | | **JLS/IAEC-01/01/10/2015** | | | | | | | | | | |
| **Set I (Working Standard)** | | | **2044.11 IU** | | | | | | | | | | |
| **Dilution details: Potency 2044.11 IU/vial** | | | 2044.11 IU/Vial dissolved in 50ml (40.88IU/ml) --->10.00ml--->make upto 25.55ml (16.00 IU/ml) --> 10ml--->make up to 20ml, (8.00 IU/ml) 10ml--> make upto 20ml (4.00 IU/ml). | | | | | | | | | | |
| **W.Std. B.No: WS/CHG05** | | | | | | | | | | | | | |
| **Date of Mfg:** | | **20/05/2015** | | | **Date of Expiry:** | | | **20/05/2016** | | | | | |
| **Time of buffer preparation:** | | | 11.30 A.M. | | **Daily dose of injection:** | | | 0.5 ml | | | | | |
| **Time of injection:** | | | 12.45 P.M. | | | | | | | | | | |
| **Buffer Lot.No.** | | | JLS/01/10/2015 | | | | | | | | | | |
| **ANIMAL DISTRIBUTION AND DISSECTION DETAILS** | | | | | | | | | | | | | |
| **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** |
| | | **Initial** | **Final** | | | | **Initial** | **Final** | | | | **Initial** | **Final** | |
| 4.0 IU | H | 31 | 38 | 27 | 8.0 IU | H | 31 | 40 | 39 | 16.0 IU | H | 31 | 42 | 52 |
| | B | 31 | 40 | 32 | | B | 32 | 41 | 40 | | B | 31 | 44 | 55 |
| | T | 31 | 39 | 26 | | T | 32 | 42 | 36 | | T | 32 | 42 | 53 |
| | HB | 33 | 42 | 30 | | HB | 33 | 45 | 38 | | HB | 35 | 43 | 55 |
| | BT | 35 | 44 | 30 | | BT | 35 | 45 | 37 | | BT | 35 | 45 | 55 |
| | HBT | - | - | - | | HBT | - | | - | | HBT | - | - | - |
| | HT | - | - | - | | HT | - | - | - | | HT | - | - | - |
| | A | - | - | - | | A | - | - | - | | α | - | - | - |
| | Total Wt | | | **145** | | Total Wt | | | **190** | | Total Wt | | | **270** |
| Cage No | | | | 1 | Cage No | | | | 2 | Cage No | | | | 3 |

| | | | | |
|---|---|---|---|---|
| **Set II (Sample Single Assay)** | **26000** IU | | | |
| **Diln. details: Potency 13000 IU/mg** | 26000 IU × 10 mg dissolved in 200 ml (1300.00 IU/ml) →10.00 ml→ make upto 100 ml (130.00 IU/ml) 10 ml→ 81.25 ml (16.00 IU/ml) 10 ml→ make upto 20 ml (8.00 IU/ml)10 ml→ make upto 20 ml (4.00 IU/ml) | | | |
| **Time of buffer preparation:** | 11.30 A.M. | **Daily dose of injection:** | 0.5 ml | |
| **Time of injection:** | 12.45 P.M. | | | |
| **Buffer Lot.No.** | JLS/01/10/2015 | | | |

| **ANIMAL DISTRIBUTION AND DISSECTION DETAILS** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** |
| | | **Initial** | **Final** | | | | **Initial** | **Final** | | | | **Initial** | **Final** | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4.0 IU | H | 30 | 41 | 28 | 8.0 IU | H | 31 | 40 | 40 | 16.0 IU | H | 31 | 40 | 55 |
| | B | 32 | 43 | 25 | | B | 31 | 40 | 36 | | B | 32 | 42 | 52 |
| | T | 34 | 41 | 30 | | T | 34 | 40 | 40 | | T | 32 | 44 | 55 |
| | HB | 35 | 45 | 29 | | HB | 35 | 43 | 37 | | HB | 34 | 44 | 58 |
| | BT | 35 | 44 | 27 | | BT | 36 | 45 | 34 | | BT | 36 | 46 | 57 |
| | HBT | - | - | - | | HBT | - | | - | | HBT | - | - | - |
| | HT | - | - | - | | HT | - | - | - | | HT | - | - | - |
| | A | - | - | - | | α | - | - | - | | α | - | - | - |
| | Total Wt | | | **139** | | Total Wt | | | **187** | | Total Wt | | | **277** |
| Cage No | | | | 4 | Cage No | | | | 5 | Cage No | | | | 6 |

**DUPLICATE ASSAY**

| | | | |
|---|---|---|---|
| **Set III (Sample Duplicate Assay)** | **26000** IU | | |
| **Diln. details: Potency 13000 IU/mg** | 26000 IU × 10 mg dissolved in 200 ml (1300.00 IU/ml) →10.00 ml→ make upto 100 ml (130.00 IU/ml) 10 ml→ 81.25 ml (16.00 IU/ml) 10 ml→ make upto 20 ml (8.00 IU/ml)10 ml→ make upto 20 ml (4.00 IU/ml) | | |
| **Time of buffer preparation:** | 11.30 A.M. | **Daily dose of injection:** | 0.5 ml |
| **Time of injection:** | 12.45 P.M. | | |
| **Buffer Lot.No.** | JLS/01/10/2015 | | |

**ANIMAL DISTRIBUTION AND DISSECTION DETAILS**

| **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Initial** | **Final** | | | | **Initial** | **Final** | | | | Initial | Final | |
| 4.0 IU | H | 31 | 40 | 26 | 8.0 IU | H | 30 | 42 | 40 | 16.0 IU | H | 30 | 39 | 52 |
| | B | 32 | 42 | 32 | | B | 30 | 42 | 36 | | B | 33 | 41 | 56 |
| | T | 33 | 43 | 29 | | T | 34 | 43 | 37 | | T | 33 | 46 | 57 |
| | HB | 35 | 46 | 31 | | HB | 35 | 46 | 41 | | HB | 35 | 46 | 54 |
| | BT | 35 | 46 | 27 | | BT | 36 | 46 | 39 | | BT | 35 | 46 | 51 |
| | HBT | - | - | - | | HBT | - | | - | | HBT | - | - | - |
| | HT | - | - | - | | HT | - | - | - | | HT | - | - | - |
| | A | - | - | - | | α | - | - | - | | α | - | - | - |
| | Total Wt | | | **145** | | Total Wt | | | **193** | | Total Wt | | | **270** |
| Cage No | | | | 7 | Cage No | | | | 8 | Cage No | | | | 9 |

**BLANK ASSAY**

| | | | |
|---|---|---|---|
| **Set IV (Blank)** | | | |
| **Phosphate buffer** | | | |
| **Time of buffer preparation:** | 10.12 A.M. | **Daily dose of injection:** | 0.5 ml |
| **Time of injection:** | 11.06 A.M. | | |
| **Buffer Lot.No.** | JLS/01/11/2014 | | |

**ANIMAL DISTRIBUTION AND DISSECTION DETAILS**

| **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** | **Total Dose** | **I.D. Mark** | **Body weight (in gms)** | | **Wt of seminal vesicles (in mg)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Initial** | **Final** | | | | **Initial** | **Final** | | | | **Initial** | **Final** | |
| 2.0 ml | H | 30 | 40 | 11 | | H | | | | | H | | | |
| | B | 31 | 40 | 10 | | B | | | | | B | | | |
| | T | 32 | 42 | 13 | | T | | | | | T | | | |
| | HB | 35 | 46 | 11 | | HB | | | | | HB | | | |
| | BT | 36 | 46 | 13 | | BT | | | | | BT | | | |
| | HBT | - | - | - | | HBT | - | - | | | HBT | - | - | - |
| | HT | - | - | - | | HT | - | - | - | | HT | - | - | - |
| | A | - | - | - | | A | - | - | - | | α | - | - | - |
| | Total Wt | | | **58** | | Total Wt | | | **0** | | Total Wt | | | **0** |
| Cage No | | | | 10 | Cage No | | | | 11 | Cage No | | | | 12 |

Where H= Head, B= Back, T= Tail, HB= Head Back, BT=Back Tail, HT=Head Tail, α= Without Mark

**Table 2: Calculation of potency**

| | | | |
|---|---|---|---|
| Ta = Ts – Tstd | | | T1 = Std low |
| Tstd = T1+T2+T3 | | | T2 = Std medium |
| Ts = T4+T5+T6 | | | T3 = Std high |
| Tb = (T3+T6) - (T1+T4) | | | T4 = Sample low |
| i* = Interval between successive log doses of std preparation | | | |
| And spl preparation | | | T5 = Sample medium |
| M= 4i* X Ta/3Tb + log R | | Anti log M= | T6 = Sample high |
| R = Vstd / Vs | | | Std – Standard |
| Potency = Anti log M | S – Sample | | |
| Average potency = potency of single assay + potency of duplicate assay / 2 | | | |
| Assay % = Average potency X 100 / Label claim potency | | | |
| **Set I (Single Assay)** | | **Set II (Duplicate Assay)** | |
| Ta = | -2 | Ta = | 3 |
| Tb = | 263 | Tb = | 250 |
| Tstd= | 605 | Tstd= | 605 |
| Ts = | 603 | Ts = | 608 |
| I = | 0.30103 | I = | 0.30103 |
| M = | 4.41192 | M = | 4.419 |
| Anti-log M= | 25817.91 | Anti-log M= | 26289.95 |
| Potency = | 25817.91IU/vial | Potency = | 26289.95IU/vial |
| **Avg. Potency = 26053.93 IU/mg** | | | |
| **Assay (%) = 100.2 % of Limit of Assay** | | | |

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