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Research Article

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Purification of hCG hormone using ion exchange chromatography

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ABSTRACT

In the present study, a higher purity of 1500 IU/mg was obtained with the crude hCG extract which was subjected to a second round of solvent fractionation to extract 12.32 mg of protein with purification. Virus deactivation step was performed here. To purify, column chromatography was used which gave 8.12 mg of protein content with 1500 IU/mg of biological activity.

Key words: hCG extract, Column chromatography, solvent fractionation, 1500IU/mg

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INTRODUCTION

The identification and accurate clinical information and interpretation of the diseases involve the identification of the biomarker. hCG family represents one such biomarker [1] and hence used in the detection and monitoring pregnancy. Qualitative estimation of hCG is of importance in the detection of pregnancy loss and ectopic pregnancy [2]. hCG is a biomarker for trophobalstic diseases, cancer prognosis and down syndrome [3,4,5] During the normal menstrual cycle [6] a very low levels of hCG have been reported. Markers that confirm pregnancy are found both in urine and blood. The hCG as a markers for pregnancy was discovered in 1930 [7], produced by the syncytiotrophoblast cells of the fertilized ova / eggs. hCG can be detected only after implantation [9]. In continuation of our earlier work [10], a higher purity of 1500 IU/mg was obtained in the present study.

EXPERIMENTAL SECTION

The process of extraction was similar to that discussed in our earlier work [10].The crude hCG obtained from the previous steps is dissolved in distilled water on ice. Using the ortho phosphoric acid, the crude solution was maintained at p^H 4.2. It was then transferred into cooling centrifuge and is subjected to 9500rpm for 30mins. The supernatant was collected and stored at 4^0c. The supernatant from the above step is allowed to pass through an ultra filtration by dissolving in distilled water at 50rpm until the conductivity of 3.5-5 µm filters are achieved. The p^H of the solution was adjusted to 4.2 and is stored at 4^0c. DEAE Anion exchanger resin was used for further purification. The product obtained was maintained at p^H 4.2-6.0 with sodium hydroxide. Thereafter, the sample is passed through the sodium acetate and glycine buffers.

RESULTS AND DISCUSSION

The crude hCG extract was subjected to solvent fractionation to extract 12.32 mg of protein with purification, the virus deactivation step was performed here. Later on, all the steps used were to obtain high purity hCG. DEAE column chromatography gave 8.12mg of protein content was obtained with 1500 IU/mg of biological activity (Table 1). The purity was assured dividing the experimental setup into 4 sets. Set-1 is the working standard with dilution details of 2044.11 IU/Vial --->50ml (40.88 IU/ml), 10.00--->51.10ml (8.00IU/ml)---> 10ml--->20ml (4.00IU/ml)--->10ml--->20ml (2.00IU/ml). With a daily dose of injection of 0.5ml, the total dose injected into cage-1, cage-2 & cage-3 were 4.0IU, 8.0IU, 16.0IU respectively. The initial and the final body weights of the rats were recorded in gm on first and fifth day of experiment and the weight of the seminal vesicles in mg on fifth day. Set-2, sample single assay with a dilution potency of 1500IU/mg×10mg--->100ml (150IU/ml), 10.00ml--->187.5ml (8.00IU/ml) --->10ml--->20ml (4.00IU/ml) --->10ml--->20ml (2.00IU/ml). HCG was injected daily (0.5ml) and the total dose was 4.0IU, 8.0IU, 16.0IU in cage-4, cage-5, cage-6 respectively. In the above, the initial and final body weights were noted. Simultaneously the weight of the seminal vesicles was also recorded. Set-3, sample duplicate assay (Table 2) was similar to set-2 and was performed with rats in cages-7,8,9. These readings were assessed against the blank assay (Table 3), maintained as set-4 and in the cage-10. The buffer used was the phosphate buffer. The initial and the final body weights of the rats were recorded in gm on first and fifth day of experiment and the weight of the seminal vesicles in mg on fifth day. The final potency was calculated (Table 4). A higher purity of 1500 IU/mg was thus obtained in the present study.

TABLE 1 : BIOASSAY RESULTS – 1500IU/mg PURIFICATION

Product Name:	**HCG -1500 IU/mg (B.P.)**		
Batch No:	***PHD002***	**Date of analysis:**	02.11.2014
Reg. No:	**JLSR/11/01001/14**	**Date of completion:**	06.11.2014
Label claim:	**1500 IU /mg**	**Date of report:**	10.11.2014
Date of Mfg:	**Oct' 2014**		
Protocol No.	**JLS/IAEC-01/01/11/2014**		

Set I (Working Standard)	**2044.11**	**IU**	X	1	vial	→	50	Ml	(40.88 IU/ml)
Dilution details: Potency 2044.11 IU/vial		10.00 ml→ 51.10 ml (8.00 IU/ml) → 10 ml→ 20 ml (4.00 IU/ml) → 10 ml→ 20 ml (2.00 IU/ml)							
W.Std. B.No: WS/CHG05									
Date of Mfg:	**20/05/2014**	**Date of Expiry:**			**20/05/2015**				
Time of buffer preparation:		10.12 A.M.		**Daily dose of injection:**		0.5 ml			
Time of injection:		10.45 A.M.							
Buffer Lot.No.									

ANIMAL DISTRIBUTION & DISSECTION DETAILS

TotalDose	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	39	26	8.0 IU	H	30	40	38	16.0 IU	H	30	41	51
	B	31	41	31		B	32	42	41		B	30	43	56
	T	32	43	27		T	33	43	35		T	33	43	54
	HB	33	45	32		HB	34	46	39		HB	34	45	57
	BT	36	46	29		BT	35	46	37		BT	35	46	52
	HBT	-	-	-		HBT	-		-		HBT	-	-	-
	HT	-	-	-		HT	-	-	-		HT	-	-	-
	α	-	-	-		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)	**1500**	IU	X	10	mg	100	ml	(150.00IU/ml)
Diln. details: Potency 1500IU/mg	10.00 ml→187.5 ml (8.00 IU/ml) → 10 ml→ 20 ml (4.00 IU/ml) → 10 ml→ 20 ml (2.00 IU/ml)							
Time of buffer preparation:	10.12 A.M.	**Daily dose of injection:**	0.5 ml					
Time of injection:	10.51 A.M.							
Buffer Lot.No.	**JLS/01/11/2014**							

ANIMAL DISTRIBUTION & DISSECTION DETAILS													
TotalDose	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	30	39	39	16.0 IU	H	30	40	56
	B	33	43	25		B	31	40	36		B	32	42	51
	T	34	43	31		T	34	40	40		T	32	44	55
	HB	35	45	29		HB	35	43	37		HB	34	44	59
	BT	36	45	27		BT	36	45	34		BT	36	46	57
	HBT	-	-	-		HBT	-		-		HBT	-	-	-
	HT	-	-	-		HT	-	-	-		HT	-	-	-
	α	-	-	-		A	-	-	-		α	-	-	-
	Total Wt			**140**		Total Wt			**186**		Total Wt			**278**
Cage No				4	Cage No				5	Cage No				6

TABLE 2: DUPLICATE ASSAY- 1500IU/mg PURIFICATION

Set III (Sample Duplicate Assay)	**1500**	IU	X	10	mg		100	ml	(150.00 IU/ml)
Diln. details: Potency 1500 IU/mg	10.00 ml→ 187.5 ml (8.00 IU/ml) → 10 ml→ 20 ml (4.00 IU/ml) → 10 ml→ 20 ml (2.00 IU/ml)								
Time of buffer preparation:	10.12 A.M.	**Daily dose of injection:**	0.5 ml						
Time of injection:	10.56 A.M.								
Buffer Lot.No.	JLS/01/11/2014								

ANIMAL DISTRIBUTION & DISSECTION DETAILS													
TotalDose	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	41	16.0 IU	H	30	39	51
	B	32	42	33		B	30	42	36		B	33	41	55
	T	33	43	30		T	34	43	39		T	33	46	57
	HB	35	46	32		HB	35	46	42		HB	35	46	53
	BT	35	46	28		BT	36	46	37		BT	35	46	50
	HBT	-	-	-		HBT	-		-		HBT	-	-	-
	HT	-	-	-		HT	-	-	-		HT	-	-	-
	α	-	-	-		A	-	-	-		α	-	-	-
	Total Wt			**149**		Total Wt			**195**		Total Wt			**266**
Cage No				7	Cage No				8	Cage No				9

Table 3: BLANK ASSAY-1500IU/mg PURIFICATION

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 & DISSECTION DETAILS

TotalDose	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	32	41	14		H					H			
	B	33	41	9		B					B			
	T	34	43	13		T					T			
	HB	35	45	10		HB					HB			
	BT	36	46	12		BT					BT			
	HBT	-	-	-		HBT	-	-			HBT	-	-	-
	HT	-	-	-		HT	-	-	-		HT	-	-	-
	α	-	-	-		A	-	-	-		α	-	-	-
			Total Wt	**58**				Total Wt	**0**				Total Wt	**0**
			Cage No	10				Cage No	11				Cage No	12

Table 4: CALCULATION OF POTENCY-1500IU/mg PURIFICATION

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 & 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)	
Ta = -1	
Tb = 263	
Tstd= 605	
Ts = 604	
I = 0.30103	
M = 3.17457	
Anti log M= 1494.74	
Potency = 1494.74 IU/vial	
Set II (Duplicate Assay)	
Ta = 5	
Tb = 242	
Tstd= 605	
Ts = 610	
I = 0.30103	
M = 3.18438	
Anti log M= 1528.92	
Potency = 1528.92 IU/vial	
Avg. Potency = 1511.83 IU/mg	
Assay (%) = 100.8 % of L.A	

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