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Novel Analytical Method Development and Validation for the Determination of Residual Solvents in Danazol by Gas Chromatography

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ABSTRACT:

The purpose of this research study was to develop and optimize an accurate and precise Gas Chromatography method for the determination of Residual solvents (Methanol, Ethanol, Isopropyl alcohol, Acetonitrile, Dichloromethane, Ethyl acetate, Tetrahydrofuran, Benzene, Toluene) in Danazol using the BP-624, 30 m x 0.53mm ID, 3.0 µmcolumn as stationary phase. The injection volume of samples taken is 1.2 ml with splitless injection. The temperature maintained at the injector and detector was to be 200ºC and 220ºC respectively. Nitrogen gas with flow 2.0 ml/minute used as mobile phase and the detection was by FID. The flow of hydrogen and Air was maintained at 30ml/min and 300ml/min respectively. The diluent used is Dimethyl Sulfoxide and water. All solvents were well resolved each other with diluents peak. Total run time is 24.3 min. The RTs observed for the Residual solvents Methanol, Ethanol, Isopropyl Alcohol, Acetonitrile, Dichl- -oromethane, Ethyl acetate, Tetrahydrofuran, Benzene and Toluene are 6.12, 9.40, 7.84, 9.68, 10.34, 15.78, 16.41, 17.43 & 19.80 respectively. The method was validated as meets all the regulations of System suitability, Specificity, Method Precision, Linearity, LOD & LOQ, Precision of LOQ and Accuracy/Recovery under ICH specifications.

KEY WORDS: Gas Chromatography, Residual solvents, Danazol, BP-624 stationary phase.

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INTRODUCTION:

Danazol[1], 17α-ethynyl-17β-hydroxy-4-androsteno[2,3-d]isoxazole (Fig.1). It is a derivative of the synthetic steroid Ethisterone[2], a modified testosterone, also known as 17α- ethynyl testosterone. It is used for the treatment of endometriosis.

From the literature review few methods have been reported for the determination of Danazol such as TLC[3], UV spectrophotometry[3], HPLC[4-7] and LC-MS/MS[8]. There is no reported method for the determination of Residual solvents in Danazol by Gas chromatographic method.

Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two or more phases. One of which moves continuously in a given direction and in which the individual substances exhibit different motilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size, or ionic charge density. The individual substances thus obtained can be identified or determined by analytical methods.

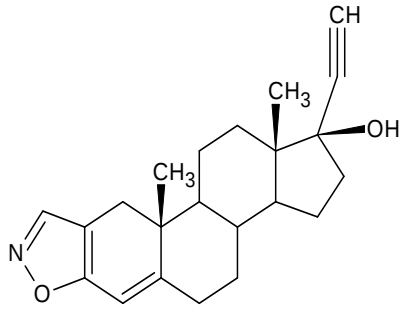

Figure.1: Structure of Danazol

Presently, in the pharmaceutical industries, special importance being given for the residual solvent testing. The residual solvents are potentially undesirable substances, they modify the properties of certain compounds and also hazardous to the health of the individual. OVI's (Organic Volatile Impurities) also affect physicochemical properties like crystallinity [9, 10] of the bulk drug, as a difference in the crystal structure may lead to change in dissolution properties and problems with formulations of the finished product. Also residual solvents may create odor problem and color change in the finished products[11,12]. The safety of the drug is determined by its pharmacological, toxicological profile and adverse effects[13,14.] The content of residual solvents in APIs are analysed by using gas chromatography[15,16].

The objective of this work is to report a simple, precise, accurate and cost effective method for the estimation of residual solvents impurities present in Danazol.

MATERIALS AND METHODS

Method Development:

Chromatographic separation was performed on a Perkin Elmer chromatographic system (Model- Clarus 500) equipped with FID detector. BP-624, 30 m x 0.53mm ID, 3.0 µm was the column used for separation. Mobile phase (carrier gas) used was Nitrogen gas with detection at 220ºc. Danazol pure drug was supplied by Dy Mach Pharma. Methanol, Ethanol, Isopropyl Alcohol, Acetonitrile, Dichloromethane, Ethyl acetate, Tetrahydrofuran, Benzene, Toluene and DMSO were of AR grade. Optimized chromatographic conditions are listed in table.1&2.

The limits for solvents were decided based on ICH guidelines. The limits of some of the solvents were kept same as per ICH guidelines while for some of the solvents the limits were decided less than the ICH guidelines depending upon the responses of those solvents obtained on the Gas chromatograph. The limits are listed in Table3.

Table.1: Optimised Chromatographic conditions

Instrument	Clarus 500
Instrument Make	Perkin Elmer
Injector Temperature	200°C
Column	30m x 0.53 mm-ID, 3.0µm BP-624
Initial Column Oven temperature	50°C
Hold time	15.0minutes
Ramp rate	30°C/min
Final Column Oven temperature	180°C
Hold time	5.0 minutes
GC Run time	24.3 minutes
Carrier gas	Nitrogen
Carrier gas flow rate	2.0 ml/min
Detector type	FID
Detector temperature	220°C
Detector Sensitivity	Range 1; Attenuation 4

Table.2: Head space parameters

Instrument	Turbomatrix 40 HS
Instrument Make	Perkin Elmer
Vial oven temperature	85°C
Vial conditioning time	for 30 minutes
Needle temperature	95°C
Transfer Line temperature	100°C
Vial Pressurisation time	for 2.0 minutes
Programmable Pneumatic Control pressure	20psi
Injection Volume	1.2 ml
Injection time	In 0.12 minutes
Cycle time	35 minutes

Table.3: Limits of solvents

Solvent	Methanol	Ethanol	IPA	ACN	DCM	EA	THF	Benzene	Toluene
Limit(ppm)	1500	5000	3000	410	600	3000	720	2	500

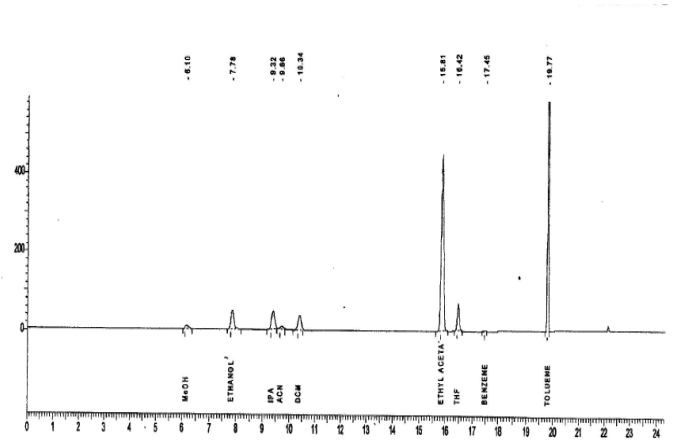

Figure 2: A Representative chromatogram for optimized method

Preparation of standard stock Solution1:

Weigh accurately about 100mg of Benzene in a 100 ml volumetric flask containing sufficient DMSO. Dilute to volume with DMSO.

Preparation of standard stock Solution2:

Weigh accurately about 205mg of Acetonitrile, 300mg of Dichloromethane, 2500mg of Ethyl Acetate, 2500mg of Ethanol, 1500mg of Isopropyl alcohol,750mg of Methanol,360mg of Tetrahydrofuran and 250mg of Toluene containing sufficient amount of DMSO. Add 1ml of Solution 1 and dilute to volume with DMSO.

Preparation of Resolution solution:

Dilute 5ml of Solution 2 to 100ml with DMSO.

Preparation of Vials:

Standard solution vial:

Pipette out 1ml of resolution solution in head space vial and add 4 ml of water and seal the vial.

Sample solution vial:

Weigh 250mg of sample in to the vial. Add 1ml of DMSO and add 4 ml of water and seal the vial.

Method Validation:

The analytical method validation was carried out as per ICH method validation guidelines. The validation parameters addressed were specificity, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), Accuracy and system suitability.

Specificity

The specificity of the analytical method was determined by injecting stock & blank solution of Dimethyl sulphoxide and water solution under the same experimental conditions. The individual Retention times of residual solvents were noted (Table 4). No peak was observed to be interfering with the solvents peaks when blank was injected.

System suitability (System precision):

Six injections were made from six separate vials of standard solution to check the precision of the system. %RSD of the six injections for all the solvents were found below 15% (Table 5).

LOD & LOQ

LODs were calculated as those concentrations that gave an S/N ratio≥ 3. LOQs were calculated as those concentrations that gave an S/N ratio≥10 and LOQ values were confirmed by checking precision of the method at LOQ level (Table 6&7).

Precision

For the method precision six replicates of mixed standard solution were injected into the chromatograph for each solvent from chromatogram peak areas standard deviation and relative standard deviation were calculated. For the precision of method and system the %RSD for six solvents complies with the acceptance criteria of less than 2%, hence the method and system is said to be précised.(Table.8)

Linearity

Prepared a series of solutions containing each solvent [i.e. LOQ level- 50%, 80%, 100%, 120% and 150% with respect to the specification limit of each solvent]. Plotted the calibration curves for each solvent at concentration ranges tested (i.e. LOQ to 150% of the specification level of each solvent) and the correlation coefficient for Residual solvents was within the limits i.e., not less than 0.99. (Table.9-11 & Fig.3-11)

Accuracy/ Recovery study

Weighed accurately about 250mg of Danazol test sample in different vials and added solution of solvent mixture with concentration of LOQ, 50%, 100% and 150% of that to the limit concentration and then added diluents as per the procedure. The % Recovery of each residual solvent should be in between 80-120 % for all four recovery levels studied (LOQ, 50, 100, and 150) of the target concentration. (Table12)

RESULTS AND DISCUSSION

All the validated parameters were found to be within the limits. Linearity is performed from LOQ to 150% and graph obtained was linear showing correlation coefficient R2 ≥0.99%. Recoveries for all solvents were found between 80-120%. System suitability for 6 injections %RSD was found to be NMT 15%.

Table.4: specificity (Individual RT's of Residual solvents)

Solvents	Retention time (min)
Methanol	6.12
Ethanol	7.84
Isopropanol	9.40
Acetonitrile	9.68
Dichloromethane	10.34
Ethyl acetate	15.78
Tetrahydrofuran	16.41
Benzene	17.43
Toluene	19.80
DMSO	N.D.

Table.5: % RSD of area's for standard solution

Sr. No	Methanol	Ethanol	IPA	ACN	DCM	Ethyl Acetate	THF	Benzene	Toluene
1	28741	174605	199742	33451	175036	1563078	195516	6003	1361238
2	29791	184268	211657	38972	182336	1615272	204023	6317	1429960
3	31082	192887	213696	31936	171287	1622490	205126	6012	1364237
4	26551	175568	196627	31754	160466	1550245	196513	5537	1246461
5	28798	182364	207302	33156	178419	1617520	203051	6336	1397061
6	27227	189284	217005	37329	184605	1693451	212225	6053	1459537
Avg	28698	183163	207668	34423	175358	1610343	202742	6043	1376416
Std.Dev	1653.08	7277.44	8050.43	2999.88	8740.91	50943.19	6137.80	289.70	74120.55
%RSD	5.76	3.97	3.88	8.71	4.98	3.16	3.03	4.79	5.39

Table.6: LOD & LOQ values

Component	Limit of Detection in ppm of the test concentration	Limit of Quantitation in ppm of the test concentration
Methanol	7.96	24.16
Ethanol	16.68	50.52
Isopropanol	12.92	39.16
Acetonitrile	3.36	10.20
Dichloromethane	2.88	8.68
Ethyl acetate	2.88	8.68
Tetrahydrofuran	2.12	6.44
Benzene	0.02	0.04
Toluene	0.56	1.64

Table.7: % RSD of areas of RS for Precision at LOQ solution

Sr. No	Methanol	Ethanol	IPA	ACN	DCM	Ethyl Acetate	THF	Benzene	Toluene
1	3217	388	2380	5009	6319	9401	5629	684	5677
2	3124	375	2516	5350	7449	10032	5979	791	6467
3	2600	353	2256	5302	5852	9101	5367	637	5344
4	2813	303	2321	5368	6647	8834	5221	712	6246
5	3192	359	2317	5331	6698	9556	5687	756	6206
6	2786	432	2495	5494	7088	9133	5429	809	6520
Avg	2955	368	2381	5309	6676	9343	5552	732	6077
Std.Dev	256.17	42.60	104.44	161.20	561.25	420.76	269.39	65.90	467.08
%RSD	8.67	11.57	4.39	3.04	8.41	4.50	4.85	9.01	7.69

Table.8: %recovery and %RSD for six injections at 100% spiking for precision study

Sr. No	Methanol	Ethanol	IPA	ACN	DCM	Ethyl Acetate	THF	Benzene	Toluene
1	106.28	101.32	99.32	113.83	98.41	95.01	96.11	92.46	93.85
2	101.69	98.78	95.52	102.85	100.38	93.82	94.52	95.55	93.14
3	110.84	102.39	99.47	108.27	96.50	95.85	97.92	90.68	92.08
4	106.30	96.81	93.17	96.64	96.26	91.31	92.11	96.58	94.40
5	96.80	100.52	98.66	103.68	96.87	96.51	98.80	93.22	93.53
6	104.98	95.02	92.51	102.37	95.35	91.89	92.99	90.71	91.87
Avg	104.48	99.14	96.44	104.61	97.30	94.06	95.41	93.20	93.15
Std. Dev	4.778	2.820	3.145	5.845	1.814	2.116	2.677	2.450	1.000
%RSD	4.57	2.84	3.26	5.59	1.86	2.25	2.81	2.63	1.07

Table.9: Average Area of Methanol, Ethanol and IPA in Linearity Solution

Level	Methanol	Ethanol	Isopropanol
Level I (LOQ)	2980	372	2499
Level II (50%)	16609	95507	107320
Level III (80%)	26676	160364	179232
Level IV (100%)	32918	199143	223552
Level V (120%)	42851	244082	272869
Level VI (150%)	50968	302318	338579
Slope	88.075	163.19	303.27
Y-Intercept	1192.7	-3403.9	-2839.7
Corr. Coeff.	0.9969	0.9984	0.9998

Table.10: Average Area of Acetonitrile, DCM and Ethyl acetate in Linearity Solution

Level	Acetonitrile	DCM	Ethyl acetate
Level I (LOQ)	5220	6540	9511
Level II (50%)	18227	85952	792363
Level III (80%)	28548	149402	1353928
Level IV (100%)	35610	190877	1728076
Level V (120%)	44870	228622	2074484
Level VI (150%)	54923	283867	2587407
Slope	330.04	1261.1	2317.1
Y-Intercept	2572	-1032.2	-27678
Corr. Coeff.	0.9965	0.9990	0.9995

Table.11: Average Area of THF, Benzene and Toluene in Linearity Solution

Level	THF	Benzene	Toluene
Level I (LOQ)	5657	704	5829
Level II (50%)	101287	2816	676708
Level III (80%)	172966	4880	1173788
Level IV (100%)	219981	6148	1501012
Level V (120%)	263983	7451	1799407
Level VI (150%)	331582	10195	2232371
Slope	1224.9	13143	12043
Y-Intercept	-1692.2	-285.61	-26823
Corr. Coeff.	0.9993	0.9952	0.9993

Table 12: %Recovery of solvents for LOQ to 150% levels

Component	Recovery at LOQ level	Recovery at 50% level	Recovery at 100% level	Recovery at 150% level
Methanol	92.2	106.74	91.37	90.44
Ethanol	101.8	102.04	97.79	97.76
Isopropanol	103.04	99.78	98.17	97.76
Acetonitrile	88.27	106.57	87.85	89.81
DCM	101.25	107.34	94.17	94.39
Ethyl acetate	98.57	105.19	97.65	95.76
THF	103.69	105.48	97.44	95.62
Benzene	98.42	107.36	95.85	107.22
Toluene	105.46	109.10	95.46	94.64

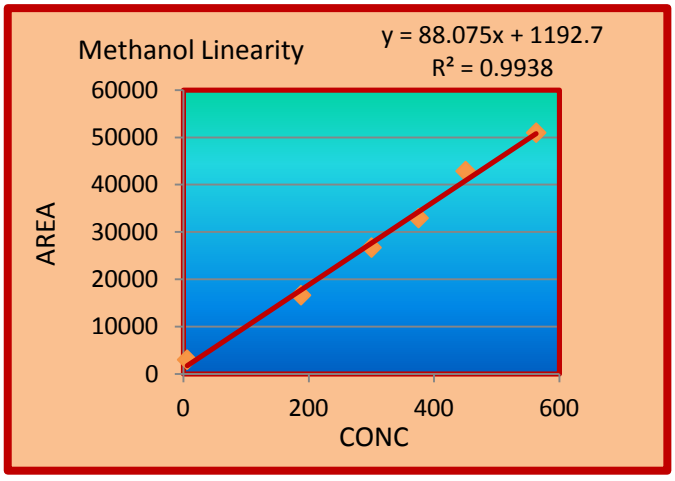

Figure.3: Linearity of Methanol

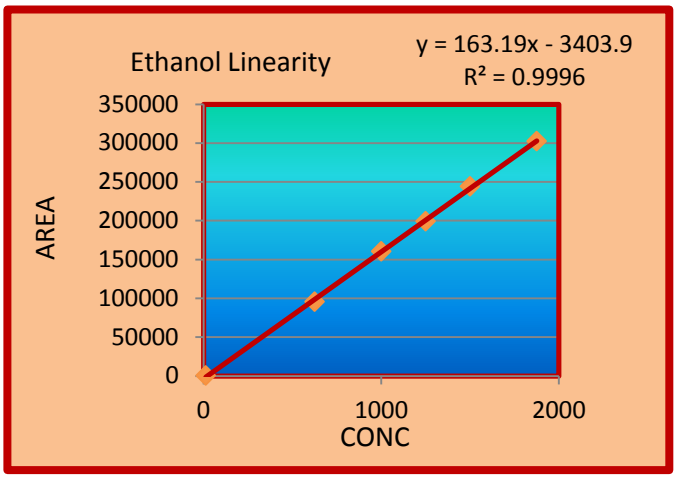

Figure.4: Linearity of ethanol

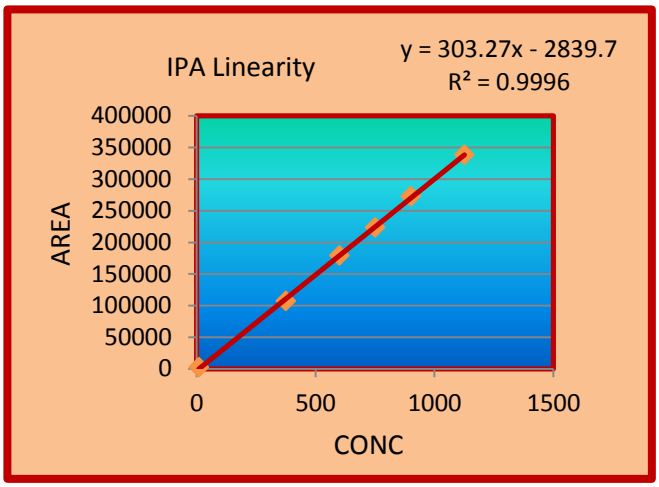

Figure.5: Linearity of IPA

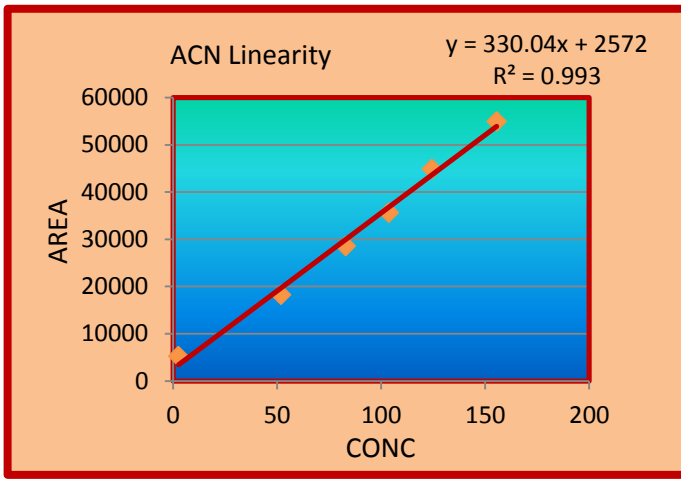

Figure 6: Linearity of Acetonitrile

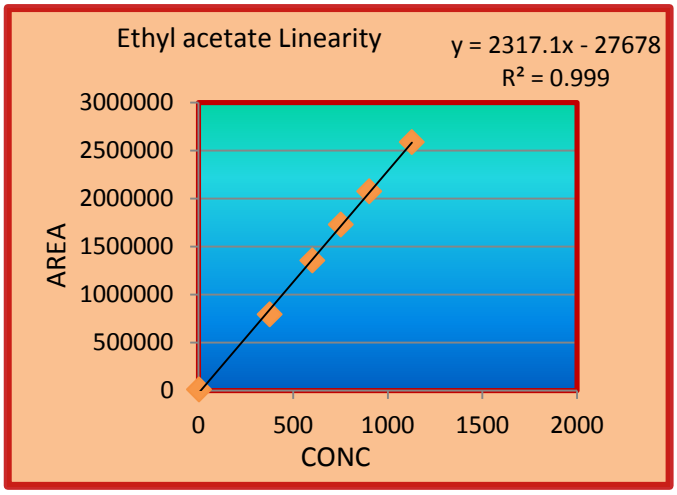

Figure 7: Linearity of Ethyl acetate

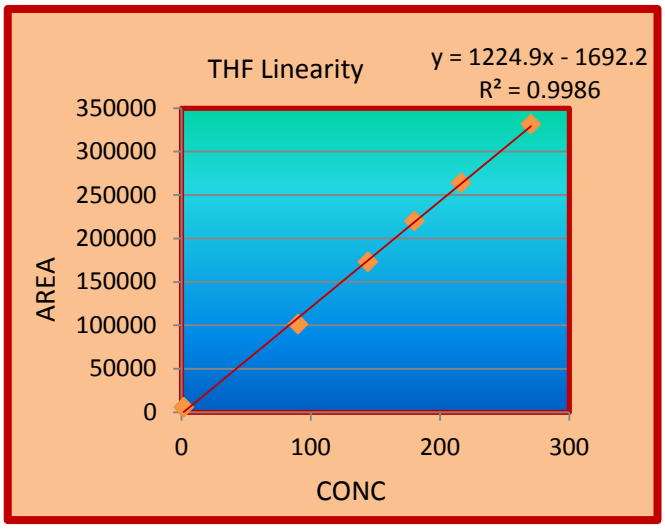

Figure 8: Linearity of Tetrahydrofuran

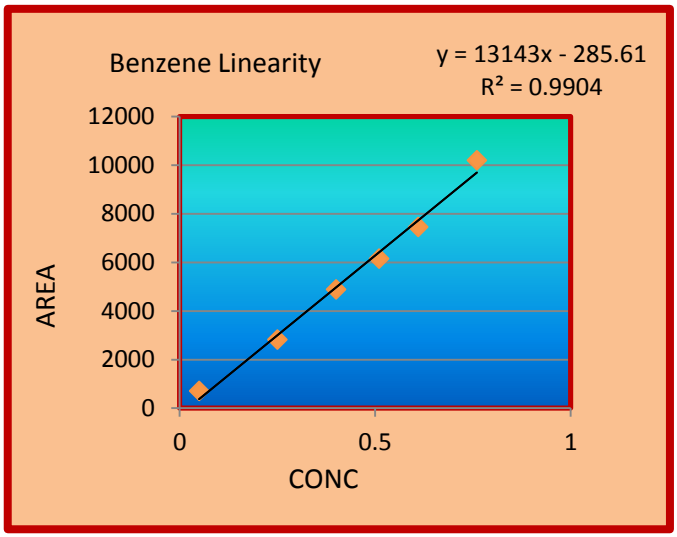

Figure 9: Linearity of Benzene

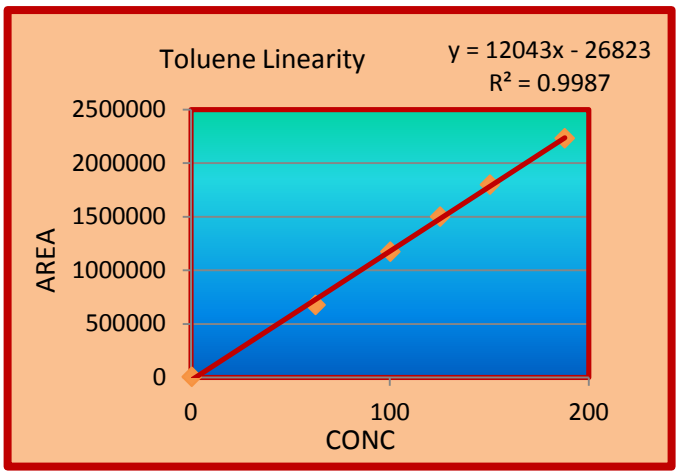

Figure 10: Linearity of Toluene

CONCLUSION

This study presents a simple and validated Gas chromatographic method for estimation of residual solvents in Danazol. The developed method is simple, specific, accurate and precise.

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