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STABILITY AND COMPATIBILITY STUDY OF DEXTROSE 5% (D5) AND DEXTROSE 5% WITH SODIUM CHLORIDE (0.9%) (DNS) INJECTION IN DIFFERENT STORAGE CONDITION.

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ABSTRACT

The purpose of the stability study is to examine, how the quality of the Active Pharmaceutical Ingredients (API) and medicinal products vary with time under different factors, such as temperature, humidity, pH and light. On the basis of the stability study, shelf life, storage condition and re-test period are determined. We carried out stability study and process validation of D5 and DNS injection in first three consecutive batches and plotted graph by using data of pH and assay of dextrose, sodium and chloride. In this study, we found degradation of dextrose in D5 and DNS injection with increasing time. Both of this parenteral products were studied accelerated and real time, and dextrose was analyzed by polarimetry, and sodium chloride was analyzed by flame photometry and titration method, finally the data were evaluated statistically.

KEYWORDS: Stability study, D5 and DNS, humidity, temperature, accelerated study, real time study.

1. INTRODUCTION

Stability of the pharmaceutical products is one of the great concern which directly co-related with quality and potency of the drug. According to the ICH and FDA guidelines, stability data are required to examine, how the environmental factors influence the quality, safety and efficacy of the drug product.[1] Pharmaceutical product having not enough stability, may change physical as well as chemical characteristic which is adversely affect to the patients. Usually, in physical changes, color and clarity of the solution, particulate matter are affected, whereas, in chemical changes, potency of the product is affected and assay percentage is

reduced.[2] As per ICH guidelines, the testing should be covered physical, chemical, biological and microbiological attributes.[3] Stability of the drug substances mainly influenced by environmental factors like humidity, temperature and light, but temperature is one of the crucial factor influence most of the drug substances. By the effect of elevated temperature, active drug substances are decomposed and efficacy is reduced as well. Study on stability helps selecting proper formulation, proper packaging of the product, to determine shelf life, and how the environmental factors influence the potency of the active substances as well as physical characteristic of the product.[4]

The indication of D5 injection is to treat hypoglycemia and fluid loss, DNS is used for the replenishment of fluid, treating minimal carbohydrate calories. Stability study on the parenteral product of D5 and DNS, helped determination of the shelf life and proper storage condition. It has been demonstrated on the basis stability data, that the actual storage condition of D5 and DNS injection is 25°C, exceeding that temperature, dextrose is decomposed to form 5-Hydroxymethylfurfural (HMF). Parenterally administration of HMF, exceeding 75mg/kg leads to adverse effects including increased activity of hepatic enzymes, altered serum-protein function and so on.[5] In this research, we performed the stability study, which is divided into two steps, real time and accelerated study. In both of this study, the first three consecutive batches of each product of D5 and DNS were evaluated. In real time study, humidity and temperature were set at 55% and 25°C respectively. Whereas, in the accelerated study, humidity and temperature were kept 75% and 40°C respectively. In case of real time study, samples were kept for the three years (shelf life of the product), and samples were analyzed every three months interval, over the first year, every six months interval over the second year and annually in the last year. Whereas, in the accelerated study, samples were kept for six months, and samples were analyzed every three months interval, up to six months. The parameters of stability studies are, Description, identification, pH, assay, particulate matter, and sterility test.

2. MATERIALS AND METHODS

D5 and DNS saline bottle purchased from local pharmacy. Cold water for injection (WFI), distilled water, 0.1(M) silver nitrate solution (AR grade) purchased from Merck, Germany.

2.1. Instruments

Digital pH meter made by Thermo Fisher Scientific, USA. Polarimeter made by Weswox, Haryana, India. Spectrophotometer made by Thermo Fisher Scientific, USA. Stability Chamber, Thermolab Scientific equipments, Maharashtra, India.

Calibration of Polarimeter

Rinsed the polarimeter tube with distilled water and filled it with distilled water, and adjusted the zero with the help of control wheel. Measured the optical rotation of blank solution. Then, prepared 10%, 20%, 30%, 40% and 50% solution in distilled water of standard, sucrose, which was previously dried at 105°C for 3 hrs. Measured the optical rotation of each sucrose solution and noted the reading.

2.2. Preparation of 0.1 (M) Silver nitrate

100ml of water was taken in a 1000 ml of cleaned and dried volumetric flask, and added 17 gm of silver nitrate with continuous stirring then added about 600 ml distilled water to mix, finally, make up volume up to 1000 ml.

2.3. Assay of Dextrose

DNS product Sample taken 50ml, diluted to 100 ml with water, measured the optical rotation of the solution. Measured the optical rotation of dextrose using polarimeter, and calculated the assay percentage of dextrose.

2.4. Assay of Sodium Chloride

DNS product sample taken 10 ml, titrate with 0.1 (M) Silver Nitrate solution. Determined the titer value and calculated the content of sodium Chloride.

2.5. Physical Characterization

DNS and D5 samples were evaluated for visual observation, pH, identification, and assay as per the guideline Indian Pharmacopeia (IP).

2.6. Stability Studies

Stability studies of the product of DNS and D5 were executed at different humidity and temperature as per ICH guideline. For the real time study, humidity and temperature were kept at 60%±5% and 25±2°C respectively, and for the accelerated study, the humidity and temperature were kept, at 75%±5% and 40°C±2°C respectively.

2.7. Microbiological test

We have carried out microbiological test for the real time and accelerated stability study, using membrane filtration method as per IP, 2014. Executed the microbiological test aseptically. The saline product of DNS and D5 were filtered through 0.45µm filter paper, separately. Teared the membrane paper, and inoculated half of the portion to Fluid thioglycollate Medium (FTGM) to support the growth of aerobic and anaerobic bacteria, and remaining half of the portion of the membrane was inoculated to Soyabean casein digest medium (SCDM) to support the growth of fungi and anaerobic bacteria. The incubation time was kept 14 days, and temperature maintained for the FTGM was 32°C (Temp. range 30 - 35°C, as per IP) and for the SCDM was 22°C (Temp. range 20 - 25°C, as per IP). We used negative and positive control as reference.

2.8. Statistical analysis

The experiment data were analyzed as the means of three replications (n=3) with standard deviation (± SD). Data were analyzed by Graph Pad Prism software and measured one-way and two-way Analysis of Variance (ANOVA) for multiple variable comparisons.

3. RESULTS AND CALCULATION

Physical Characterization of DNS and D5 has been shown in the **Table 1.**

Physical Characterization of DNS and D5 saline product.

Test	DNS	Dextrose 5% (D5)IP	Specification
Description	Clear, colorless, odorless liquid.	Clear, colorless, odorless liquid.	As per IP, 2014, (Vol. III).
pH	5.80	5.75	pH range, 5.0 -7.0 as per IP, 2014, (Vol.III).
Assay	100.5%	100.20%	As per IP, 2014.
Conductivity	1.0µs/cm	0.9µs/cm.	NMT 1.5µ Mhos at 35°C, as per IP,2014 (Vol.III).
Oxidisable substances	complies	complies	As per IP,2014 (Vol. III).
T.O.C	128 ppb	125 ppb	NMT 0.5mg/Lit. or 500 ppb, as per IP, 2014 (IP. Vol. III).
Nitrates	Complies	complies	As per IP, 2014 (Vol. III).

3.1. Assay of dextrose in DNS

50 ml, DNS sample was taken and diluted to 100 ml with distilled water, then measured the optical rotation of the solution, employed by polarimeter.

Measured optical rotation: +2.65°, +2.60°, +2.7°.

Average reading: +2.65°

Blank reading: 0.00°

Actual reading: +2.65°.

So, content of dextrose = $\frac{2.65 \times 0.9477 \times 100}{50}$

= 5.02% w/v of dextrose.

= 100.45% of claim.

3.2. Assay of Sodium Chloride in DNS

10 ml, DNS sample was taken, titrate with 0.1 (M) silver nitrate solution. Titer value was 17.0 ml, factor of 0.1(M) silver nitrate = 0.9062.

Content of Sodium chloride = $\frac{\text{Titer value x factor of 0.1 (M) silver nitrate x 0.005844 x 100}}{\text{Sampletaken}}$

$= \frac{17 \times 0.9062 \times 0.005844 \times 100}{10}$

= 0.9002% w/v

= 100.03% of claim.

3.3. Assay of Dextrose in D5

50 ml of D5 saline sample was taken, and diluted to 100 ml with distilled water, measured the optical rotation of the solution using, polarimeter.

Measured optical rotation: +2.7°, +2.7°, +2.65°

Average rotation: 2.683°

Blank reading: +0.00°

Actual reading: 2.683°

Content of Dextrose: $\frac{2.683 \times 0.9477 \times 100}{50}$

= 5.086% w/v = 101.72% of claim.

Content of sodium chloride in DNS and content of Dextrose in the DNS and D5, were decreased gradually with increment of days. The degradation rate of sodium chloride and dextrose is higher in the environmental condition of accelerated study rather than real time study. **(Fig. 3 and Fig. 4).**

3.4. Test for 5-HMF and related substances

8 ml, DNS sample was taken and diluted to 100 ml, and measured the absorbance, using spectrophotometer. The reading of the absorbance is 0.037 which complies as per IP, 2014. It indicates the quantity of the 5-HMF and other related substances in the DNS saline product is within limit.

3.5. Determination of pH

pH of DNS and D5 saline products were determined by digital pH meter, it was observed that the pH of the solution were slightly decreased gradually, with increment of days in case of both of the products. **Fig.1 and Fig.2.**

3.6. Microbiological test

Summary of the microbiological test has been shown in **Table 2.**

Test	Medium	Tube No.	Observation Days													Results
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	
Sample	FTGM	1	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	OK
	SCDM	2	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	OK
Negative Control	FTGM	1	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	OK
	SCDM	2	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	OK
Positive Control	FTGM	1	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	OK
	SCDM	2	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	OK

-Ve and +Ve indicate absence and presence of the growth, respectively. No growth of fungi or bacteria observed in the saline product of DNS and D5.

3.7. Stability studies

The real time and accelerated studies were carried out in different environmental conditions of RH and temperature as specified in IP, 2014. Determination of pH and assay of both of this product were analyzed in different time point and described the summary in the following table.

Summary of the real time and accelerated study has been shown in the Table 3:

			DNS			D5	
	Year	Frequency (Months)	pH	Assay (%)		pH	Assay (%)
				Dextrose	Sod. Chloride		
Real time study (RH: 60% ± 5%, Temp. 25°C ± 2°C)	1st year	Initial	5.78	100.43	100.20	5.75	99.45
		3	5.75	100.40	100.19	5.79	99.45
		6	5.75	100.36	100.11	5.76	99.32
		9	5.84	100.33	100.04	5.78	99.21
		12	5.88	100.28	100.00	5.81	99.12

	2nd Year	18	5.99	100.20	99.72	5.92	99.04
		24	6.09	100.14	99.45	6.13	99.00
	3rd Year	36	6.32	100.05	99.39	6.22	98.96
Accelerated Study (RH: 75% ± 5%, Temp. 40°C ± 2°C)	1st Year	Initial	5.82	100.42	100.20	5.76	99.45
		3	5.90	100.00	99.70	5.82	99.00
		6	5.95	99.42	99.10	5.83	98.27

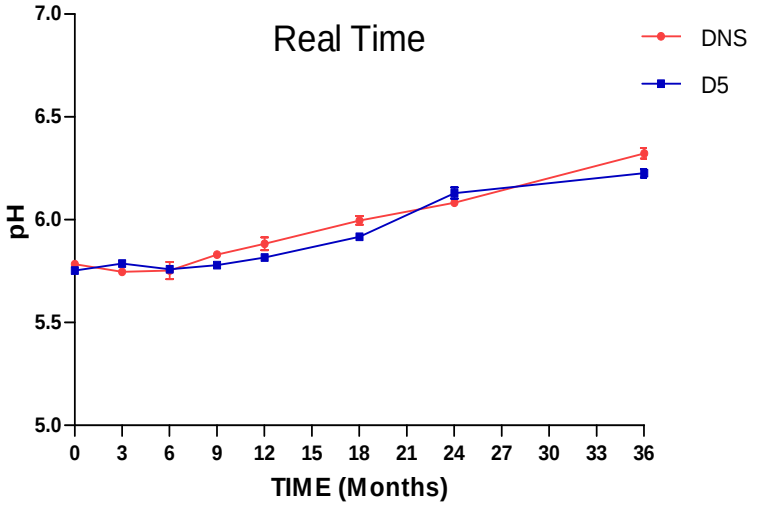

Figure 1: pH of DNS and D5 in real time study, the initial pH of DNS and D5 were 5.79 and 5.75 respectively. It was observed that pH was raised gradually with increment of days.

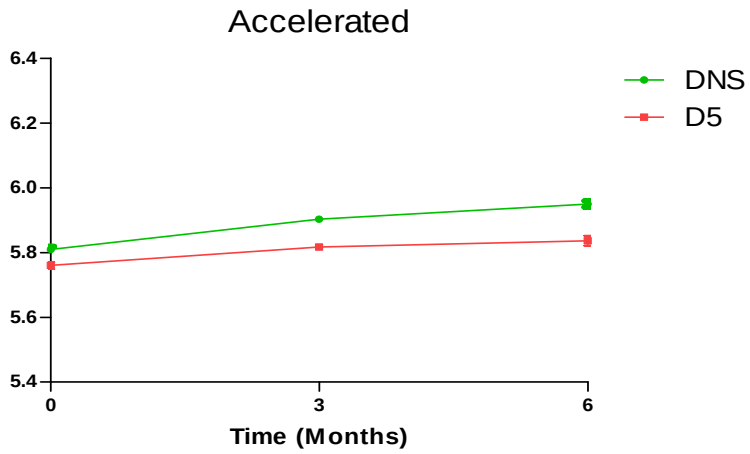

Figure 2: pH of DNS and D5 in accelerated study, the initial pH of DNS and D5 was 5.79 and 5.75. It was observed that pH was raised faster than real time study, with increment of days.

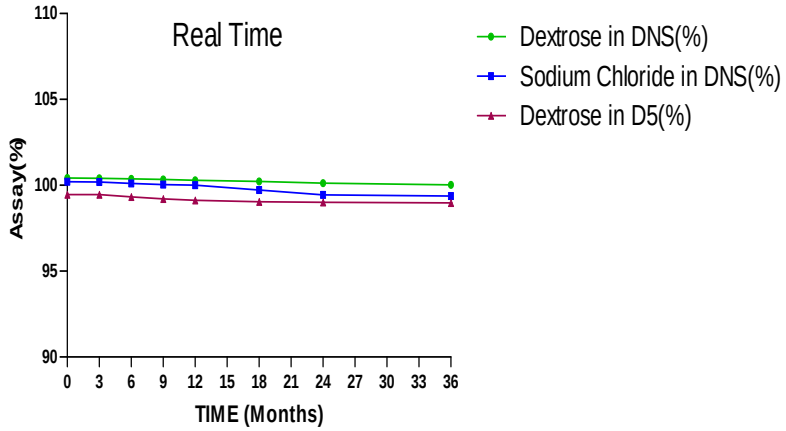

Figure 3: Real time study, assay percentage of dextrose and sodium chloride in DNS, the initial assay percentage of dextrose and sodium chloride in DNS was 100.42% and 100.20%, respectively and dextrose in D5 was 99.45%. The assay was decreased gradually with increment of days. It was observed that the assay percentage of dextrose and sodium chloride in DNS was 100.03% and 99.38, respectively, and assay percentage of dextrose in D5 was 98.96% in the 36th month after manufacturing.

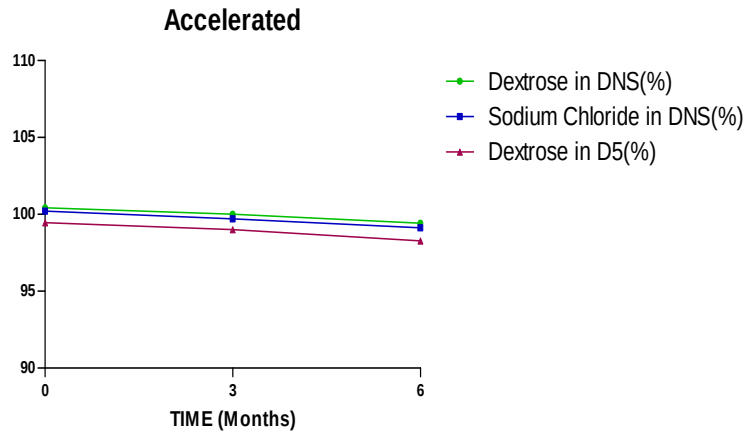

Figure 4: Accelerated study, assay percentage of dextrose and sodium chloride in DNS , the initial assay percentage of dextrose and sodium chloride in DNS was 100.42% and 100.20%, respectively and dextrose in D5 was 99.45%. The assay percentage was decreased faster in accelerated study compared with real time study, It was observed that the assay percentage of dextrose and sodium chloride in DNS was 99.42% and 99.11%, respectively, and assay percentage of dextrose in D5 was 98.27% in the 6th month after manufacturing.

3. DISCUSSION

The stability studies is carried out in consecutive three batches of new product. The main objective of the stability studies is to establish the storage condition, packaging, and shelf life of the new products, based on the analysis report of the stability study, viz. physical, chemical, biological and microbiological test. It also need to be focused that after degradation of the active product, throughout the shelf life period, assay percentage must be with in specified range as per Pharmacopeia. If the assay percentage of the active product is lowered than specified limit throughout the shelf life period, the initial assay percentage should be kept in higher range of the specified limit as per Pharmacopeia, that in no harsh condition the potency of the product will loss, lower than limit. For the photodegradable products, photo degradation study also need to be done to evaluate, the how the light effect is influenced on the potency of the products.[6] The D5 and DNS saline are not photodegradable or light sensitive products, but the D5 and DNS containing dextrose, is decomposed in higher temperature. This is the main reason to decrease the content of dextrose after terminal sterilization, and it forms 5-HMF, which possess toxic effect exceeding its limit.[7,8] We observed the content of sodium chloride and dextrose were decreased with increasing days, and the rate of decrement of assay percentage is higher in environmental condition of accelerated study compared to real time study, it signifies that the harsh condition (higher humidity and temperature zone) strongly adverse on decomposition of the active products.

4. CONCLUDING REMARKS

Stability study is the ICH and FDA approved methodologies to establish the shelf life period of a new formulation. As per the ICH guideline, we performed all of the parameters of stability study including accelerated and long term study, microbiological test, particulate matter and identification test. On the basis of our study reports, it is ensured that, the newly manufactured formulation, D5 and DNS retain its potency throughout the shelf life period, and during this time period the medicine will be safe and effective.

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