



## FORMULATION AND EVALUATION OF SULPHASALAZINE INJECTION MADE BY MIXED SOLVENCY SOLUBILIZATION TECHNIQUE

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### ABSTRACT

Sulfasalazine, a sulfonamide derivative, is used as an antimicrobial agent by inhibiting bacterial growth and activity mainly in the treatment of ulcerative colitis and in the treatment of rheumatoid arthritis. It is practically insoluble in water. The aim of study was to prepare aqueous injection of sulphasalazine, using the mixed solvency solubilization technique. Several blends were prepared by co-solvents such as polyethylene glycol 200 (PEG200), PEG 400, PEG 600, PEG 4000, propylene glycol (PG), polyvinyl pyrrolidone (PVP) K 30, ethanol, glycerin, tween20, tween80, niacinamide, lignocaine hydrochloride and sodium benzoate. The enhancement in the solubility of sulphasalazine in a mixed solvency was more than 50 folds (compared to its solubility in distilled water). This proved a synergistic enhancement in solubility of a poorly water soluble drug due to mixed solvency. Synergistic combination of mixed-solvency can minimize the amount of co-solvents employed, minimizing the chances of their toxicities. The developed formulation was studied for physical and chemical stability.

**KEYWORDS:** Mixed-solvency solubilization, aqueous injection stability, solubility enhancement, sulfasalazine

### INTRODUCTION

Poor aqueous solubility is a common concern in the pharmaceutical sciences. There are several established methods for increasing the equilibrium solubility of non-polar drugs in aqueous vehicles co-solvency, the addition of water miscible solvents to an aqueous system, is one of the oldest, most powerful, and most popular of these<sup>1</sup>. Co-solvent solubilization is particularly important for parenteral dosage forms where it is desirable to incorporate the required dose as a true solution in the smallest volume of liquid as possible. Co-solvents are used in 13% of FDA approved parenteral products, and the co-solvents<sup>2,3,4</sup>.

Most co-solvents have hydrogen bond donor and/or acceptor groups as well as small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility while their hydrophobic hydrocarbon regions interfere with water's hydrogen bonding network, reducing the overall intermolecular attraction of water. By disrupting water's self-association, co-solvents reduce water's ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility. A different perspective is that by simply making the polar water environment more non-polar like the solute, co-solvents facilitate solubilization. This is supported by the observation that co-solvents reduce the solubility of polar compounds such as amino acids, ostensibly by reducing the polarity of the aqueous environment and thereby reducing the favorable interactions between solute and solvent<sup>1</sup>. The use of co-solvents has been employed by a number of workers to enhance the solubility of poorly soluble drugs<sup>5-9</sup>.

In the present work, Sulfasalazine, a sulfonamide derivative used as an antimicrobial agent by inhibiting bacterial growth and activity mainly in the treatment of ulcerative colitis and in the treatment of rheumatoid arthritis was selected as a model drug which is a BCS class II drug (highly permeable and low soluble) and attempts were made to formulate an aqueous injection of this drug, using mixed solvency. The formulation was also studied for physical and chemical stability<sup>10</sup>.

### MATERIALS AND METHODS

#### Materials

Sulphasalazine was obtained from as a gift sample from Ronak Life Care pvt Ltd, Patan, India. Sodium benzoate, niacinamide, polyethylene glycol, lignocaine hydrochloride, ethanol, tween, ascorbic acid, and poly vinyl pyrrolidone were purchased from Central Chemicals Mumbai, India. All other chemical and solvents were of analytical grade and freshly prepared distilled water was used throughout study.

#### Methods<sup>1,5,8,9,15,16</sup>

#### Preparation of blends

Blends were prepared in distilled water. Accurately weighed solubilizing agents were transferred in to small amount of distilled water and completely dissolved with the help of magnetic stirrer. Volume was made up with distilled water up to 100 ml. Table 1 shows all these information about blend preparation.

#### Preparation of calibration curve in different medium

For preparation of calibration curve in de-mineralized water, blends, buffers of pH 7.4, 8.0, 9.0 (Physiological pH and pH range of blends) and different mixed solvency blends: 10 mg of sulphasalazine was weighed accurately and transferred to 100 ml volumetric flask. Drug was dissolved and the volume was made up to 100 ml with the respective medium so as to obtain stock solutions of 100 µg/ml. Appropriate dilutions from the stock solutions were made with the de-mineralized water to give solution containing 10, 20, 30, 40, 50, 60, and 70µg/ml. The absorbance of the resulting drug solutions were read on UV spectrophotometer (Shimadzu® 1700) at 359 nm against the respective blank. The data were recorded in Table 2.

#### Optimization of blend for preparation of injection

On the basis of solubility data given in Table 5, three blends were selected. The volume of hydrotropic blend solution required for the formulation of injection in each case was determined. Formulation-1 was prepared by 2%w/v sulphasalazine in blend-1, where formulation were 2& 3

prepared by 1.5%w/v sulphasalazine in blend 15 & blend 18 respectively.

#### **Formulation of aqueous injection:**

##### **1. Preparation of blend**

Every ingredient for each blend as mentioned in table 5 were accurately weighed and transferred into 100ml volumetric flask. 60-70ml of water for injection was added to the flask and flask was shaken to dissolve the contents. Then volume was made up to 100ml.

##### **2. Preparation of aqueous solution of sulphasalazine**

For the preparation of aqueous solution of sulphasalazine, about 35 ml of hydrotropic blends solution was taken in 50 ml of volumetric flask. Weighed amount of sulphasalazine was transferred into volumetric flask and the flask was sonicated until complete dissolution of drug. Volume was made up to 50 ml with same hydrotropic blend solution. Then content of volumetric flask were sonicated for additional 20 min on the sonicator for complete solubilization and equilibration. The initial solution was diluted 2 times with WFI and sonicated for 5 min for proper mixing of WFI and contents. This solution was stable for at least 12 hr at refrigerated condition.

##### **3. Treatment of packaging material**

Glass vials of 10 ml capacity were washed several times with distilled water. All these vials were dried and sterilized by dry heat in oven at 160°C for 2 hr. in inverted position. Rubber plugs used for plugging the vials were first washed several times with distilled water and then autoclaved at 15 lbs pressure (120°C) for 20 min and finally dried in vacuum oven.

##### **4. Preparation of aseptic area**

The walls and floor of aseptic room was thoroughly cleaned and then disinfected with 5% phenol solution. Aseptic room was fumigated by using 40% v/v formaldehyde solution prepared in distilled water. Fumigation was allowed to carry out overnight by keeping the prepared solution on heating mantle settled on 20°C. The laminar airflow bench was cleaned and wiped out with 70% ethanol solution and switched on UV light was for 30 min prior to filling of injection into vials.

##### **5. Aseptic filtration**

The aqueous solution of sulphasalazine was prepared as above and sterilized by filtration under the pressure through 0.22 µm disposable membrane filter, fitted in filtration assembly of 500 ml glass bottle. Whole assembly was sterilized by autoclaving at 15 lbs pressure for 15min.

#### **EVALUATION<sup>11-16</sup>**

##### **Determination of interference of solubilizing agents in the spectrophotometric estimation of drug**

For determination of interference of solubilizing agents in the spectrophotometric estimation of sulphasalazine, the absorbances of the standard solutions of drugs were determined in de-mineralized water alone and in the presence of the maximum concentration of the solubilizing agents employed for spectrophotometric analysis/formulation purpose in the present investigation.

##### **Determinations of equilibrium solubility**

For equilibrium solubility determination at room temperature, excess solute method was employed. Sufficient excess amounts of drugs were added to screw capped 10 ml glass vials containing de-mineralized water, solutions of solubilizing agents, solution of mixed solvency blends and buffers 7.4, 8.0 and 9 separately. The vials were shaken

mechanically for 12 h at room temperature in orbital flask shaker (Khera Instruments Pvt. Limited, Delhi, India). The solutions were allowed to equilibrate for next 24 h and the solutions were transferred into tubes then centrifuged for 5 min at 2000 rpm using a centrifuge (Remi Instruments Limited, Mumbai, India). The supernatants of each vial were filtered through Whatman filter paper #41. Filtrates of saturated solutions of sulphasalazine were analyzed by spectrophotometric analysis using single beam UV visible spectrophotometer, measuring the absorbance of appropriately diluted solutions (with de-mineralized water)/respective buffers against respective reagent blanks at 359 nm wavelengths. Solubilities so determined have been shown in Table 4. Enhancement ratios (Table 4.) in solubilities were determined by following formula:

$$\text{Enhancement ratio} = \frac{\text{Solubility of drug in hydrotropic solution}}{\text{Solubility of drug in demineralised water}}$$

#### **Stability studies**

##### **Physical stability study of formulated injection of sulphasalazine**

The vials were subjected to physical stability studies by keeping the vials at different temperatures conditions. A control sample was observed for 30 days for colour, pH, and precipitation. Physical stability of sulphasalazine is given in Table 6.

##### **Chemical stability study of formulated injection of sulphasalazine**

The vials were subjected to stability studies by keeping the vials at different temperature and humidity conditions. A control sample was kept at refrigerated conditions. The amount of sulphasalazine was estimated by UV spectroscopy method at time interval of 15 days and 30 days and was expressed in terms of % drug remaining. The initial drug content was taken to be 100%.

##### **Stability study with anti-oxidants**

For determination of formulations stability studies two anti-oxidants likes' ascorbic acid and sodium sulfide were selected. The vials were subjected to stability studies by keeping the vials at different temperatures conditions. A control sample was observed for 30 days for colour, pH and precipitation. Stability study with anti-oxidants is given in Table 8.

#### **RESULTS**

##### **Determination of interference of solubilizing agents in the spectrophotometric estimation of drug:**

It was found that sulfasalazine having no interference with different solubilizing agent. Result was shown in table 4.

##### **Determinations of equilibrium solubility**

Maximum solubility of sulfasalazine was found to be in blend1, blend 15 and blend 18. Result was shown in table 5.

#### **Stability studies**

##### **Physical stability study of formulated injection of sulphasalazine:**

All formulations were found to be stable and results were shown in table 6.

##### **Chemical stability study of formulated injection of sulfasalazine:**

All formulations were found to be stable and results were shown in table 7.

##### **Stability study with anti-oxidants**

All formulations were found to be stable and results were shown in table 8.

## DISCUSSION

The objective of the present research is to explore the application of mixed solvency solubilization technique in the formulation of dosage forms of water-insoluble drugs and to reduce concentration of individual solubilizing agents to minimize the side effects. In solubilization, a high concentration of an additive is required to produce an appreciable increase in aqueous solubility of a poorly water-soluble drug. In this case, the solubilizing agent employed to give a desirable solubility may produce its own toxicity. However, if the same enhancement in solubility can be achieved by mixing, say five solubilizers (each in one fifth concentration) then the toxic level of the five solubilizers can be greatly reduced. In case of synergistic effect in solubility due to mixing of, say, five solubilizers (in one fifth concentration), the toxic level of individual solubilizers can further be lowered because still less concentration of the solubilizing agents shall be sufficient for a desired enhancement in solubility.

For determination of interference of solubilizing agents the spectrophotometric estimation of sulphasalazine, the absorbance of the standard solutions of drug were determined in distilled water alone and in the presence of the maximum concentration of the solubilizing agents employed for spectrophotometric analysis and formulation purpose in the present investigation and UV spectrum of solubilizing agents were also recorded and concluded that there is no interference of solubilizers in the spectrophotometric analysis of sulphasalazine.

Calibration curves of sulphasalazine were prepared in distilled water, different blends (as solubilizing agent) and buffers of pH 7.4, 8.0 and 9.0 (corresponding pH of blends).

Equilibrium solubility of a sulphasalazine in different media was determined by excess solute method and the solubility enhancement ratios were calculated. From the results of the solubility data it was concluded that the aqueous solubility of sulphasalazine was increased more than 40 times. It is concluded that the solubility of sulphasalazine increases synergistically by mixed solvency. The stability of the sulphasalazine bulk solution was established in process water for a period of 12 hours, which showed that the bulk solution was stable during the time required for filling operations.

Physical stability study of formulated injection of sulphasalazine was performed. The results showed that the injection was physically stable.

Physical stability studies with different anti-oxidants give valuable information.

1. Ascorbic acid is not suitable for aqueous injection of sulphasalazine because it leads to precipitation with sulphasalazine.
2. Sodium sulfide gives good result.
3. Plain aqueous injection of sulphasalazine gives good result as compared with anti-oxidants so no need to add anti-oxidants in aqueous injection of sulphasalazine.

The above research findings showed that, a stable aqueous injection formulations containing sulphasalazine were successfully developed. There is good scope for other poorly water-soluble drugs to develop their aqueous formulation by the use of combination of suitable solubilizers at reduced concentration. The proposed solubilizers are known to be safe hence, toxicities/safety related issues may not arise, suggesting the adoptability for large scale manufacturing i.e. industrial feasibility. The proposed techniques would be economical, convenient and safe. Thus, the study opens the chances of preparing such aqueous formulation of poorly-water soluble drugs.

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Table 1: Preparation of various mixed solvency blends (in %)

Blend	PEG 200	PEG 400	PEG 600	PEG 4000	Ethanol	Glycerine	Niacina mide	Benzyl alcohol	Lignocain HCL	Na benzoate	Poly-ethylene glycol	PVP K 30	Tween 20	Tween 80
B1	-	5	-	-	5	-	-	-	-	5	5	5	-	3
B2	-	5	4	-	5	4	-	-	-	-	4	-	4	-
B3	-	4	4	4	-	-	5	-	-	4	-	3	-	-
B4	-	4	4	-	4	4	5	-	-	-	-	-	-	4
B5	-	4	-	-	-	4	5	-	-	4	4	-	4	-
B6	-	5	-	5	-	-	5	-	-	4	5	4	-	-
B7	-	-	-	4	5	5	5	-	-	-	-	-	3	3
B8	-	-	5	-	5	-	5	-	-	5	-	4	-	3
B9	-	4	-	-	4	4	-	-	-	4	4	4	-	-
B10	-	4	4	4	-	-	-	-	-	-	4	-	4	4
B11	-	4	4	4	4	4	-	-	-	-	-	-	2	2
B12	-	4	4	-	-	3	2	-	-	2	4	-	2	2
B13	-	-	-	5	-	5	5	-	-	5	-	4	-	-
B14	-	-	-	5	4	2	5	-	-	5	-	4	-	-
B15	-	-	-	4	2	2	5	-	-	5	-	4	2	2
B17	-	2	2	4	2	2	4	-	-	2	2	2	2	2
B18	-	-	-	7	-	-	7	-	1	7	-	4	-	-
B19	5	-	-	5	-	-	4	-	2	4	-	-	-	-
B20	4	-	-	-	-	-	4	2	2	4	-	-	4	-
B21	4	-	-	-	-	-	4	-	2	2	-	4	-	4
B22	2	2	-	2	2	2	2	2	2	2	2	2	2	2

Table 2: Calibration curve of sulphasalazine in different blends

Sr. No.	Blend use	Y=mX+c equation	R <sup>2</sup> value
1	Bland-1	Y=0.050x+0.014	0.985
2	Bland-2	Y=0.050x+0.017	0.978
3	Bland-3	Y=0.049x+0.017	0.984
4	Bland-4	Y=0.050x+0.023	0.964
5	Bland-5	Y=0.050x+0.024	0.966
6	Bland-6	Y=0.041x+0.020	0.982
7	Bland-7	Y=0.041x+0.023	0.982
8	Bland-8	Y=0.053x+0.023	0.978
9	Bland-9	Y=0.042x+0.027	0.980
10	Bland-10	Y=0.039x+0.019	0.986
11	Bland-11	Y=0.045x+0.017	0.987
12	Bland-12	Y=0.045x+0.023	0.986
13	Bland-13	Y=0.048x+0.031	0.968
14	Bland-14	Y=0.039x+0.019	0.987
15	Bland-15	Y=0.041x+0.025	0.982
16	Bland-16	Y=0.034x+0.016	0.989
17	Bland-17	Y=0.052x+0.013	0.988
18	Bland-18	Y=0.041x+0.027	0.978
19	Bland-19	Y=0.041x+0.032	0.971
20	Bland-20	Y=0.039x+0.029	0.971
21	Bland-21	Y=0.041x+0.015	0.992
22	Bland-22	Y=0.046x+0.028	0.977
23	Phosphate buffer pH7.4	Y=0.042x+0.041	0.958
24	Phosphate buffer pH8.0	Y=0.038x+0.016	0.990
25	Phosphate buffer pH 9.0	Y=0.039x+0.018	0.990

Table 3: Optimized formula

Sr. No.	Ingredients	Formulation-1	Formulation-2	Formulation-3
1.	sulphasalazine	2 g	1.5 g	1.5 g
2.	PEG 400	5 g	-	-
3.	Ethanol	5 g	2 g	-
4.	Propylene glycol	5 g	-	-
5.	Sodium benzoate	5 g	4 g	7 g
6.	Tween 80	3 g	2 g	-
7.	Niacenamide	-	5 g	7 g
8.	PEG 4000	--	4 g	7 g
9.	PVP	5 g	4 g	4 g
10.	Glycerine	-	2 g	-
11.	Tween 20	-	2 g	-
12.	Lignocaine HCL	0.5 g	0.5 g	1 g
13.	Water for injection (q.s.)	100 ml	100 ml	100 ml

Table 4: Interference studies of solubilizing agents

Sr.No.	Drug	Solvent System	Concentration of drug used ( $\mu\text{g/ml}$ )	$\lambda_{\text{max}}$ (nm)	Absorbance against respective blank
1	Sulphasalazine	DM water	5	359	0.312
2	Sulphasalazine	Blend 2	5	359	0.321
3	Sulphasalazine	Blend 4	5	359	0.312
4	Sulphasalazine	Blend 5	5	359	.0311
5	Sulphasalazine	Blend 6	5	359	0.320
6	Sulphasalazine	Blend 7	5	359	0.322
7	Sulphasalazine	Blend 8	5	359	0.315
8	Sulphasalazine	Blend 9	5	359	0.324
9	Sulphasalazine	Blend 10	5	359	0.326
10	Sulphasalazine	Blend 11	5	359	0.300
11	Sulphasalazine	Blend 12	5	359	0.317
12	Sulphasalazine	Blend 13	5	359	0.329
13	Sulphasalazine	Blend 14	5	359	0.330
14	Sulphasalazine	Blend 15	5	359	0.315
15	Sulphasalazine	Blend 16	5	359	0.315
16	Sulphasalazine	Blend 17	5	359	0.316
17	Sulphasalazine	Blend 18	5	359	0.314
18	Sulphasalazine	Blend 19	5	359	0.336
19	Sulphasalazine	Blend 20	5	359	0.329
20	Sulphasalazine	Blend21	5	359	0.339
21	Sulphasalazine	Blend 22	5	359	0.348

Table 5: Equilibrium solubility of sulphasalazine in different media

Sr. No.	Solvent	pH of solvent system	Solubility* (%)	Solubility Enhancement ratio
1	DM water	7.2	0.1358	-
2	Blend 1	7.6	8.41	61.92
3	Blend 2	7.8	3.87	28.49
4	Blend 3	7.4	3.25	23.93
5	Blend 4	8.8	0.50	3.68
6	Blend 5	9.0	2.62	19.29
7	Blend 6	9.4	3.06	22.53
8	Blend 7	8.4	3.90	28.71
9	Blend 8	7.6	4.00	29.45
10	Blend 9	8.6	2.95	21.72
11	Blend 10	7.6	3.56	26.21
12	Blend 11	8.4	3.33	24.52
13	Blend 12	9.0	2.56	18.85
14	Blend 13	7.8	2.87	21.13
15	Blend 14	8.6	3.29	24.22
16	Blend 15	9.0	5.34	39.32
17	Blend 16	9.2	3.62	26.65
18	Blend 17	8.8	2.94	21.64
19	Blend 18	8.6	5.54	40.79
20	Blend 19	8.4	3.14	23.12
21	Blend 20	7.6	3.54	26.06
22	Blend 21	7.8	3.66	26.95
23	Blend 22	8.2	2.94	21.64
24	Phosphate buffer	7.4	3.62	26.65
25	Phosphate buffer	8.0	3.65	26.85
26	Alkaline borate buffer	9.0	4.00	29.45

\* Average of 3 determinations

Table 6: Physical stability of sulphasalazine injection

Formulation	Conditions	Physical stability parameter					
		pH		Colour		Precipitation	
		Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
1	Refrigeration (2-8°C)	7.62	7.85	Orange	Orange	No ppt.	No ppt.
1	Room Temperature	7.62	7.75	Orange	Orange	No ppt.	No ppt.
1	40°C/75% RH	7.64	7.55	Orange	Orange	No ppt.	No ppt.
1	55°C	7.62	7.89	Orange	Orange	No ppt.	No ppt.
2	Refrigeration (2-8°C)	9.00	9.02	Orange	Orange	No ppt.	No ppt.
2	Room Temperature	9.00	9.25	Orange	Orange	No ppt.	No ppt.
2	40°C/75% RH	9.00	9.26	Orange	Orange	No ppt.	No ppt.
2	55°C	9.00	9.32	Orange	Orange	No ppt.	No ppt.
3	Refrigeration (2-8°C)	8.60	8.68	Orange	Orange	No ppt.	No ppt.
3	Room Temperature	8.60	8.71	Orange	Orange	No ppt.	No ppt.
3	40°C/75% RH	8.60	8.68	Orange	Orange	No ppt.	No ppt.
3	55°C	8.60	8.88	Orange	Orange	No ppt.	No ppt.



Table 7: chemical stability of sulphasalazine injection

Time (days)	Formulation	% Residual drug		
		Room temperature	40°C/75% RH	55°C
0	Formulation-1	100.00	100.00	100.00
	Formulation-2	100.00	100.00	100.00
	Formulation-3	100.00	100.00	100.00
15	Formulation-1	99.87	99.33	99.17
	Formulation-2	99.33	98.28	98.82
	Formulation-3	99.28	98.87	98.87
30	Formulation-1	99.33	97.85	98.85
	Formulation-2	98.67	97.52	98.05
	Formulation-3	98.45	97.65	98.27

Table 8: Stability study with anti-oxidant

Formulation	Conditions	Physical stability parameter					
		pH		Colour		Precipitation	
		Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
Plain 1	Refrigeration (2-8°C)	7.60	7.88	Orange	Orange	No ppt.	No ppt.
Plain 1	Room Temperature	7.65	7.77	Orange	Orange	No ppt.	No ppt.
Plain 1	40°C/75% RH	7.71	7.78	Orange	Orange	No ppt.	No ppt.
Plain 1	55°C	7.72	7.89	Orange	Orange	No ppt.	No ppt.
Plain 2	Refrigeration (2-8°C)	9.08	9.12	Orange	Orange	No ppt.	No ppt.
Plain 2	Room Temperature	9.20	9.25	Orange	Orange	No ppt.	No ppt.
Plain 2	40°C/75% RH	9.09	9.28	Orange	Orange	No ppt.	No ppt.
Plain 2	55°C	9.04	9.32	Orange	Orange	No ppt.	No ppt.
Plain 3	Refrigeration (2-8°C)	8.67	8.78	Orange	Orange	No ppt.	No ppt.
Plain 3	Room Temperature	8.69	8.75	Orange	Orange	No ppt.	No ppt.
Plain 3	40°C/75% RH	8.68	8.78	Orange	Orange	No ppt.	No ppt.
Plain 3	55°C	8.70	8.78	Orange	Orange	No ppt.	No ppt.
Ascorbic acid 1	Refrigeration (2-8°C)	6.72	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 1	Room Temperature	7.52	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 1	40°C/75% RH	7.44	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 1	55°C	7.22	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 2	Refrigeration (2-8°C)	8.00	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 2	Room Temperature	8.50	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 2	40°C/75% RH	7.90	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 2	55°C	7.50	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 3	Refrigeration (2-8°C)	8.60	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 3	Room Temperature	7.68	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 3	40°C/75% RH	7.80	NR	Orange	Black	No ppt.	Ppt.
Ascorbic acid 3	55°C	7.15	NR	Orange	Black	No ppt.	Ppt.
Sodium sulfide 1	Refrigeration (2-8°C)	8.22	8.85	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 1	Room Temperature	81.52	8.75	Orange	Light black	No ppt.	No ppt.
Sodium sulfide 1	40°C/75% RH	8.64	8.85	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 1	55°C	8.72	8.90	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 2	Refrigeration (2-8°C)	9.10	9.22	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 2	Room Temperature	9.09	9.15	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 2	40°C/75% RH	9.12	9.76	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 2	55°C	9.00	9.52	Orange	Light black	No ppt.	No ppt.
Sodium sulfide 3	Refrigeration (2-8°C)	8.60	8.88	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 3	Room Temperature	8.60	8.91	Orange	Orange	No ppt.	No ppt.
Sodium sulfide 3	40°C/75% RH	8.60	NR	Orange	Black	No ppt.	Ppt.
Sodium sulfide 3	55°C	8.60	NR	Orange	Black	No ppt.	Ppt.

\* NR = Not responding, Ppt. = Precipitate

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