APPENDIX B

sanofi aventis

Because health matters

Industrial Analytical Development - Great Valley	
Analytical Study Report	**Doc # IAD-060626-ELX**

Analytical Assessment of Oxaliplatin Solutions Containing Tartaric Acid

(Patent Application # WO 2005/020980 A1)

<Part or all of the information in this document may be unpublished material and should be treated as the confidential property of sanofi-aventis. The use of such information or material must be restricted to the recipient from the agreed purpose and must not be disclosed to any unauthorized persons, in any form, including publications and presentations, without the written consent of sanofi-aventis. >

Table of Content

1. OBJECTIVE 3

2. ANALYTICAL APPROACH 3

3. MATERIALS 3

4. EXPERIMENTAL PROCEDURE 3

4.1. PREPARATION OF TEST AND CONTROL SOLUTIONS 3
4.2 STABILITY TESTING 5

5. ANALYTICAL RESULTS AND DISCUSSION 5

5.1 ANALYTICAL RESULTS FOR pH 4.0 6
5.2 ANALYTICAL RESULTS FOR pH 5.0 8
5.3 ANALYTICAL DATA SUMMARY FOR DAY 7 10
5.4 MASS SPECTROMETRY DATA 11

6. CONCLUSION 13

Appendix A- Study Protocol 14

Analytical Assessment of Oxaliplatin Solutions Containing Tartaric Acid

(Patent Application # WO 2005/020980 A1)

1. Objective

The objective of this study is to assess the possible formation of tartaroplatin through ligand exchange of oxalic acid and tartaric acid in Oxaliplatin solutions containing tartaric acid. The analytical approach for this study is documented in the attached protocol (Doc# IAD-060530-ELX). This report describes the analytical study results.

2. Analytical Approach

Lab scale Oxaliplatin solutions containing (+)-tartaric acid were prepared according to patent application number 2005/020980 A1 titled "Acid Containing Oxaliplatin Formulation". Three tartaric acid concentrations, 0.2, 3.0, and 6.7 mM, were assessed at pH 4.0 and 5.0. The solutions were tested for the presence and formation of Tartaroplatin. Oxaliplatin aqueous solution was used as analytical control.

3. Materials

3.1 Oxaliplatin drug substance: Lot# LO02T089, manufactured by Tanaka Kikinzoku Kogyo K.K.
3.2 (+)-tartaric acid: Sigma Aldrich, A.C.S. reagent, ≥ 99.5%, Lot# 02316AA
3.3 Water: HPLC grade, J.T. Baker, Lot# B28E53
3.4 NaOH solution: 1N, EMD, Lot# 4345
NaOH Solution: 6N, J.T. Baker, Lot# A50517
3.5 Tartaroplatin reference solution:
Molecular Profiles, UK, provided the tartaroplatin reference solution shipped on dry ice. The solutions were in frozen condition upon receipt. The tartaroplatin reference solutions were stored at – 20°C prior to analysis. This solution served as a retention time marker for the tartaroplatin peak formation through ligand exchange in this study.

4. Experimental Procedure

4.1. Preparation of Test and Control Solutions

Lab scale Oxaliplatin solutions containing 0.2, 3.0, and 6.7 mM of tartaric acid at target pH 4.0 and 5.0 were prepared. The composition of the solutions is listed below:

Oxaliplatin concentration: 5 mg/mL
Tartaric acid concentrations: 0.2, 3.0, and 6.7 mM
Water: Water For Injection (WFI)
pH: 4.0 (range 3.7 – 4.3) and 5.0 (range 4.7 – 5.3)

The following example demonstrates the steps for preparation of Oxaliplatin solution containing 0.2 mM of tartaric acid at pH 4.0.

As per the reference patent application, one gram of Oxaliplatin drug substance was dissolved in 200 mL of water at 50-55°C to a target concentration of 5 mg/mL. A total of 120 µL of tartaric acid solution (5% w/v) was added to the Oxaliplatin solution to achieve target tartaric acid concentration of 0.2 mM. The pH of the solution was adjusted to 4.1 using 1N sodium hydroxide solution.

Five additional Oxaliplatin solutions containing tartaric acid as listed in Table 1 were prepared in a similar procedure as the above example. The pH of each solution was adjusted with 1N or 6N sodium hydroxide solutions and recorded in Table 1.

An Oxaliplatin solution containing no tartaric acid was also prepared as an analytical control solution. The pH of this solution was measured at 5.6 and not adjusted.

Table 1. Formula Composition of Oxaliplatin Test Solutions

Tartaric acid Concentration	No Tartaric Acid 0.0 mM	I 0.2 mM		II 3.0 mM		III 6.7 mM	
Formulation ID	Control	I4.0	I5.0	II4.0	II5.0	III4.0	III5.0
Oxaliplatin (mg)	1000	1000	1000	1000	1000	1000	1000
Tartaric Acid (mg)	0	6	6	90	90	200	200
Tartaric Acid 5% w/v (µL)	0	120	120	1800	1800	4000	4000
WFI qs (mL)	200	200	200	200	200	200	200
pH	5.6	4.0	5.1	4.0	5.1	4.0	5.0

Note: molecular weight of tartaric acid = 150.09

I: formulation containing tartaric acid at 0.2mM (equivalent to 0.03 mg/mL)
II: formulation containing tartaric acid at 3.0mM (equivalent to 0.45 mg/mL)
III: formulation containing tartaric acid at 6.7mM (equivalent to 1.0 mg/mL)

4.2 Stability Testing

The solutions prepared as presented in Table 1 were stored at ambient conditions (22°C ± 2°C). Aliquots of each solution were tested at initial, 1, 3, 5 and 7 days for the possible formation of Tartaroplatin. Test and control solutions were subjected to mass spectrometry and compared with tartaroplatin reference solution.

5. Analytical Results and Discussion

The tartaroplatin peak was observed in all six preparations (I 4.0, I 5.0, II 4.0, II 5.0, III 4.0, and III 5.0) of the Oxaliplatin solutions containing tartaric acid from day 1 through day 7. The retention time of the tartaroplatin peak in those solutions matched well with the retention time of tartaroplatin reference solution received from Molecular Profiles. The tartaroplatin peak was absent in the unbuffered aqueous Oxaliplatin control solution throughout the 7 days of study. Representative chromatograms are shown Figure 1.

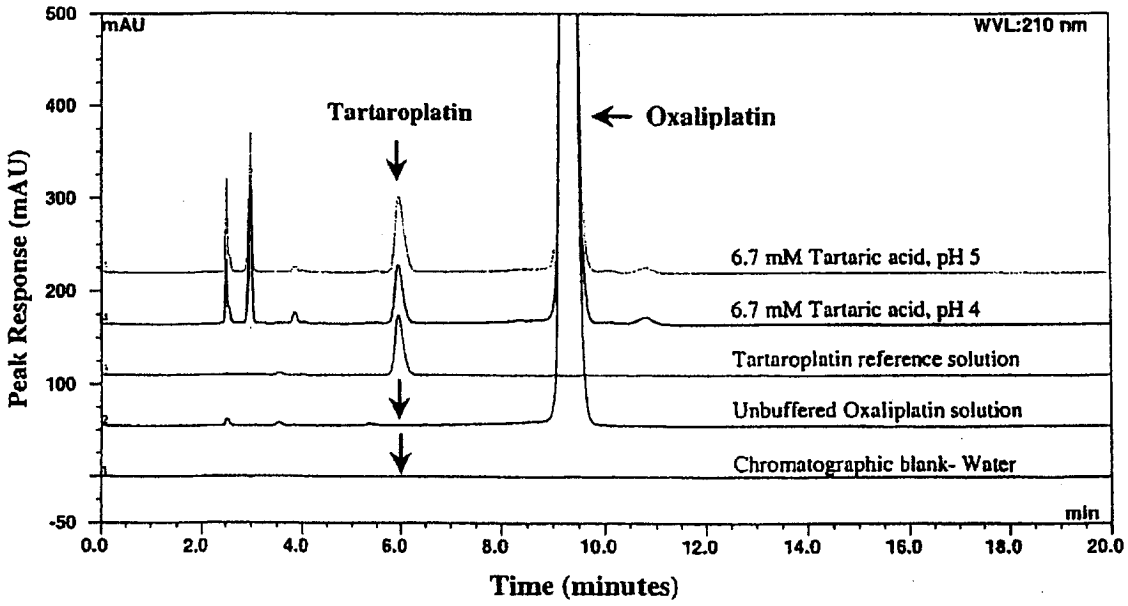

Figure 1. Representative Chromatograms of Oxaliplatin Solution Containing Tartaric Acid (III4.0 and III5.0 at 7 days) in Comparison with Tartaroplatin Reference Solution, Unbuffered Oxaliplatin Control Solution, and Chromatographic Blank (Water).

5.1 Analytical Results for pH 4.0

At pH 4.0, the increase of the tartaroplatin peak was observed in all three Oxaliplatin solutions containing tartaric acid (0.2, 3.0, 6.7 mM) from day 1 through day 7. The retention time of the tartaroplatin peak in those three experimental solutions was consistent with the reference solution provided by Molecular Profiles. The peak was absent in the unbuffered aqueous Oxaliplatin control solution throughout the 7 days. Data is summarized in Table 2.

Table 2. Tartaroplatin Peak Area and Relative Area Percentage at pH 4

Tartaric Acid Concentration	Time (Days)	Tartaroplatin Peak Area[1]	Tartaroplatin Relative Area (%)[2]
0.2 mM	1	1.0205	0.21
	3	1.6276	0.33
	5	1.7017	0.34
	7	1.8387	0.37
3.0 mM	1	4.5935	0.93
	3	6.7317	1.36
	5	8.1292	1.65
	7	9.1832	1.86
6.7 mM	1	3.2858	0.66
	3	6.7757	1.37
	5	9.4814	1.92
	7	12.1416	2.46

[1]Tartaroplatin peak area = absolute peak area recorded by HPLC
[2]Tartaroplatin relative area (%) = %Tartaroplatin peak area relative to total chromatographic peak area

As shown in Figure 2, at day 7, the peak areas of the tartaroplatin peak were relative to the concentration of tartaric acid in the solutions. The 6.7 mM solution contained the highest tartaroplatin peak response. The increases of tartaroplatin in the solutions were 8% for 0.2 mM, 13% for 3.0 mM, and 28% for the 6.7mM from day 5 to day 7 of the study. The rates of increase for the higher tartaric acid containing solutions (3.0 mM and 6.7 mM) were significant.

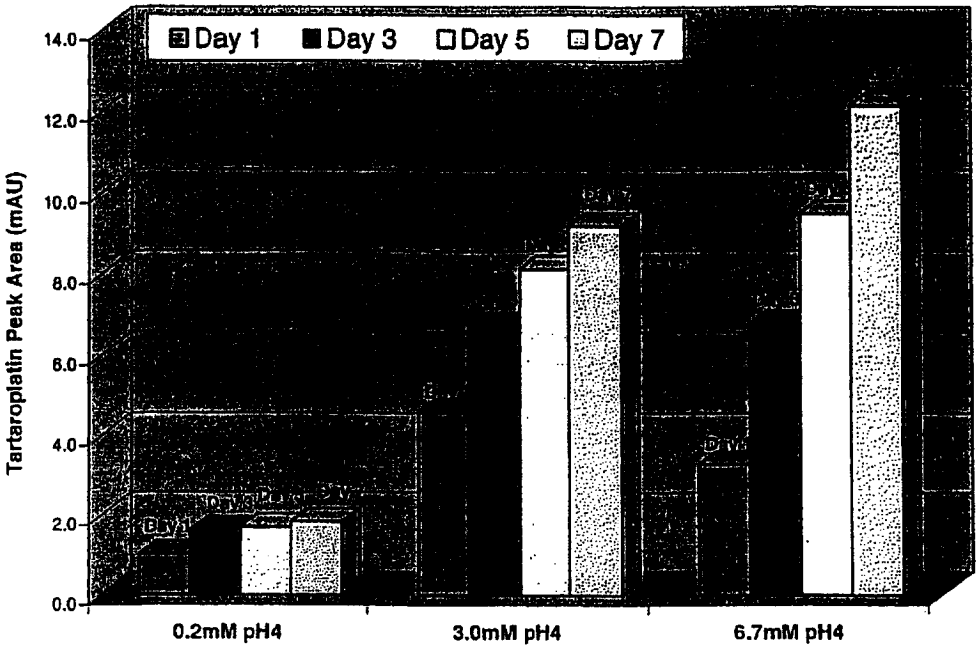

Figure 2. Tartaroplatin Peak Area Comparison of Oxaliplatin Solutions Containing Tartaric Acid at pH 4.

5.2 Analytical Results for pH 5.0

At pH 5.0, the increase of the tartaroplatin peak was observed in all three Oxaliplatin solutions containing tartaric acid (0.2, 3.0, 6.7 mM) from day 1 through day 7. The retention time of tartaroplatin peak in those three experimental solutions was consistent with the reference solution provided by Molecular Profiles. The peak is absent in the unbuffered aqueous Oxaliplatin control solution throughout the 7 days. Data is summarized in Table 3.

Table 3. Tartaroplatin Peak Area and Relative Area Percentage at pH 5

Tartaric Acid Concentration	Time (Days)	Tartaroplatin Peak Area[1]	Tartaroplatin Relative Area (%)[2]
0.2 mM	1	1.4764	0.30
	3	2.2834	0.46
	5	2.5025	0.50
	7	2.5963	0.52
3.0 mM	1	3.9646	0.80
	3	7.4228	1.50
	5	10.0415	2.04
	7	12.5816	2.54
6.7 mM	1	4.5716	0.92
	3	9.4960	1.91
	5	13.3199	2.68
	7	17.4894	3.51

[1]Tartaroplatin peak area = absolute peak area recorded by HPLC
[2]Tartaroplatin relative area (%) = %Tartaroplatin peak area relative to total chromatographic peak area

As shown in Figure 3, at day 7, the peak areas of the tartaroplatin peak were relative to the concentration of tartaric acid in the solutions. The 6.7 mM solution contained the highest tartaroplatin peak response. The increases of tartaroplatin in the solutions were 3.7% for 0.2 mM, 25% for 3.0 mM, and 31% for the 6.7mM from day 5 to day 7 of the study. The rates of increase for the higher tartaric acid containing solutions (3.0 mM and 6 mM) were significant.

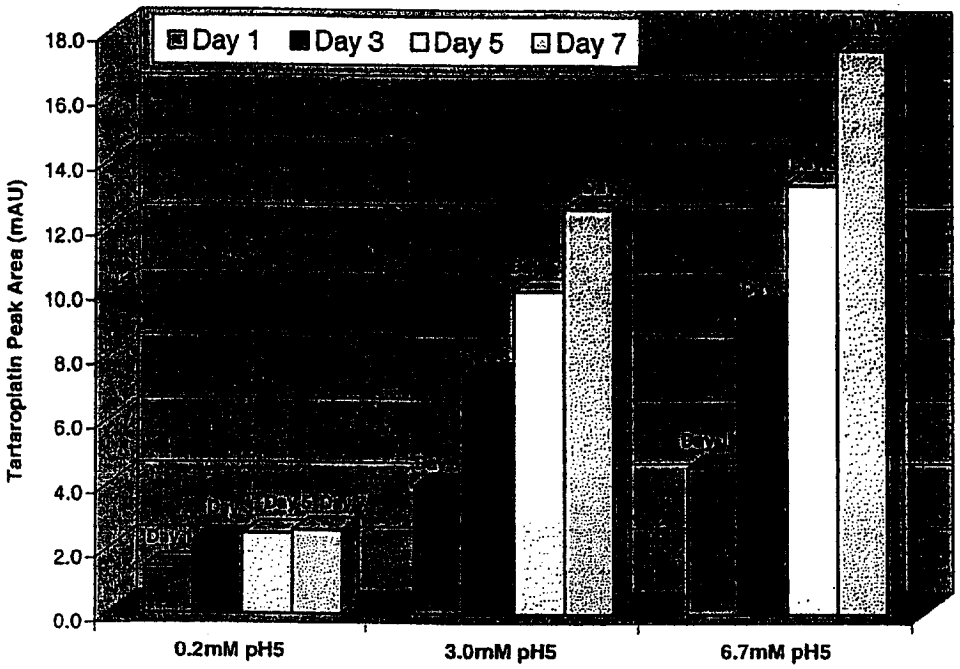

Figure 3. Tartaroplatin Peak Area Comparison of Oxaliplatin Solutions Containing Tartaric Acid at pH 5.

5.3 Analytical Data Summary for Day 7

As shown in Table 4, tartaroplatin peak responses ranged from 0.37% to 3.51% relative peak area after 7 days. The content of tartaroplatin was relative to the concentration of tartaric acid. In addition, pH 5 solutions yielded higher tartaroplatin concentrations than pH 4 solutions at the same tartaric acid concentration as indicated by Figure 4.

Table 4. Tartaroplatin Relative Peak Area (%)Comparison for Day 7

Tartaric acid Concentration	[1]Tartaroplatin Relative Area (%)	
	pH 4	pH 5
0.2 mM	0.37	0.52
3.0 mM	1.86	2.54
6.7 mM	2.46	3.51

[1]Tartaroplatin relative %area = %Tartaroplatin peak area relative to total chromatographic peak area

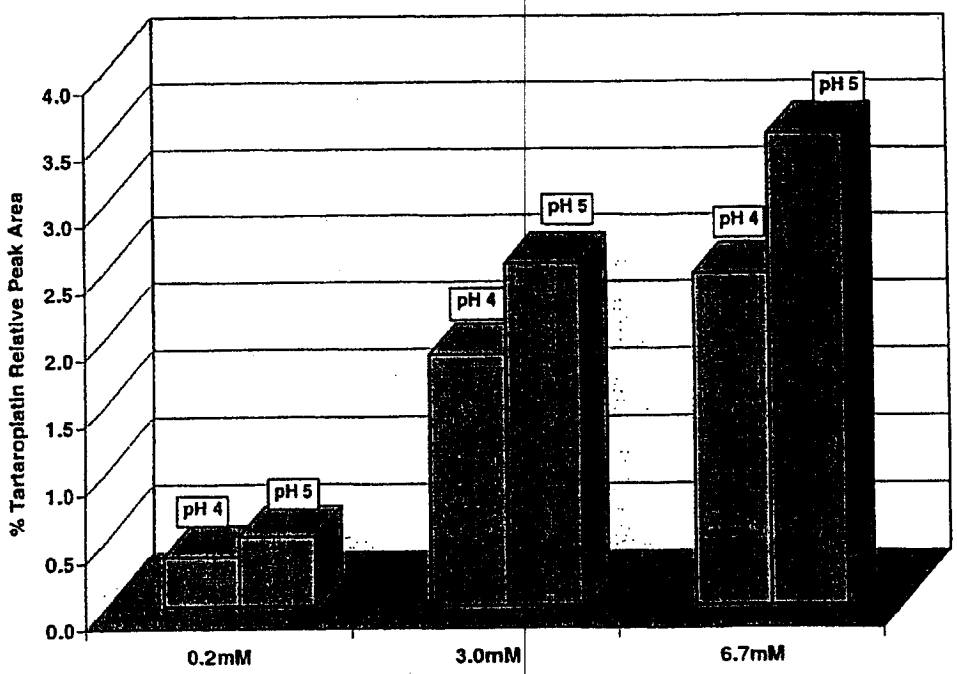

Figure 4. Day 7 Tartaroplatin Data Comparison of pH 4 and pH 5

5.4 Mass Spectrometry Data

The unbuffered Oxaliplatin control solution and the 6 test solutions were analyzed by LC/MS with electrospray mass spectrometry using the same HPLC column and mobile phase as the Tartaroplatin assay procedure. Under the experimental conditions employed for analysis, the spectra of the test solutions were consistent with the spectrum of tartaroplatin reference solution as shown in Figures 5, 6 and 7. The parent ion with a mass of 457 amu $(M+H)^+$, was present in all 6 test solutions and the tartaroplatin reference solution. The same ion was absent in the unbuffered Oxaliplatin control solution (Figure 1). This data further confirmed the formation of tartaroplatin in Oxaliplatin solutions containing tartaric acid.

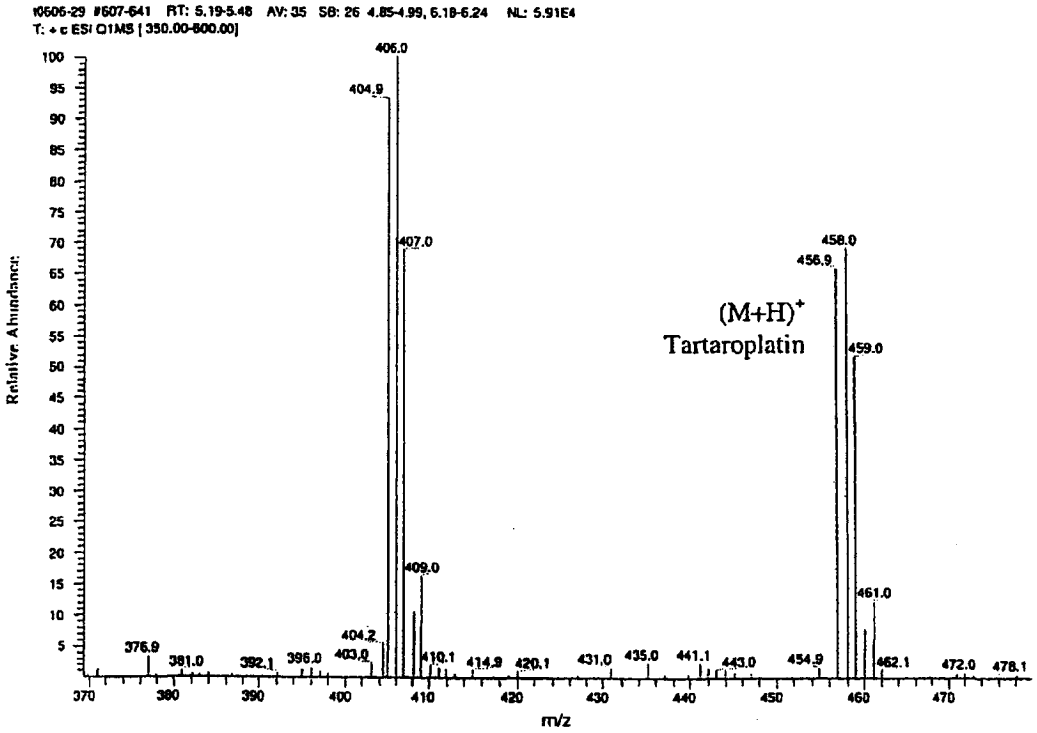

Figure 5. Spectrum of Tartaroplatin Reference Solution Received From Molecular Profiles.

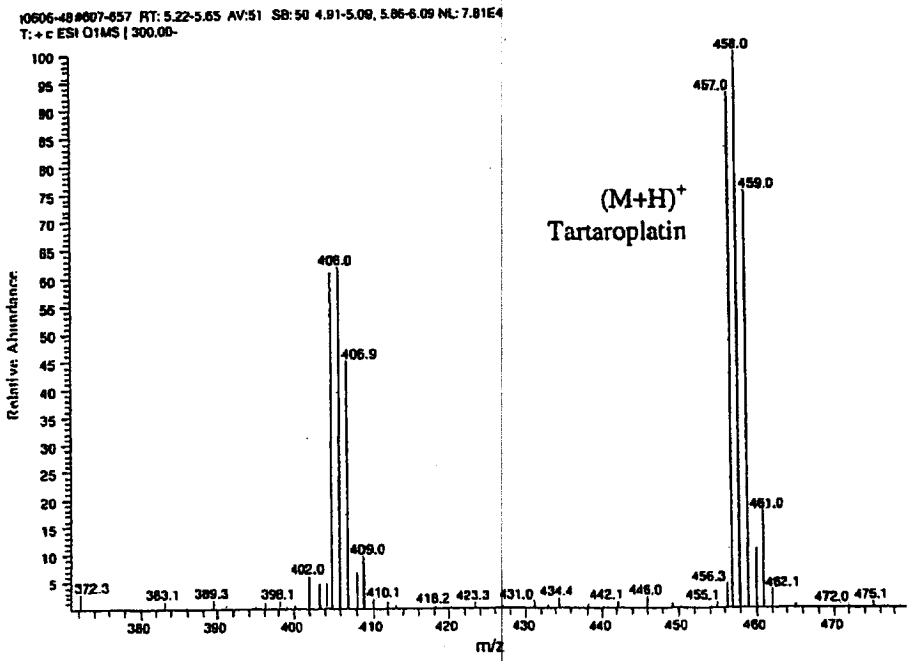

Figure 6. Spectrum of Oxaliplatin Solution Containing 6.7 mM Tartaric Acid at pH 5.

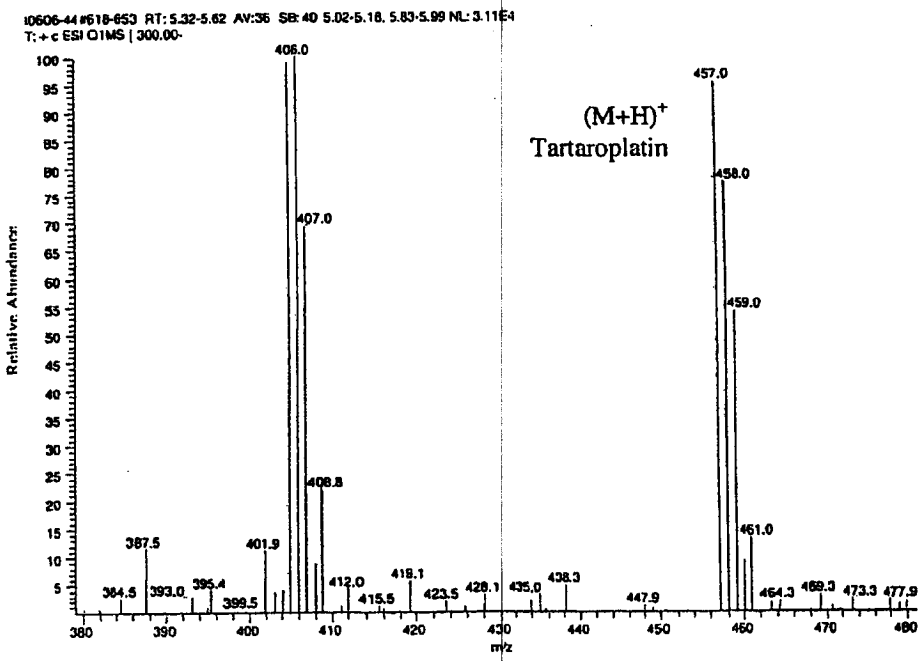

Figure 7. Spectrum of Oxaliplatin Solution Containing 0.2 mM Tartaric Acid at pH 5.

6. Conclusion

This experiment demonstrated that tartaroplatin was formed in tartaric acid containing Oxaliplatin solution through ligand exchange with oxalic acid as illustrated in Figure 8. The concentration of the tartaroplatin increased over the 7 days of the study with the highest amount detected in 6.7mM tartaric acid at pH 5. For the same tartaric acid concentration solutions, pH 5 solution contained higher tartaroplatin than pH 4 solution. This tartaroplatin peak was absent in the control unbuffered Oxaliplatin solution.

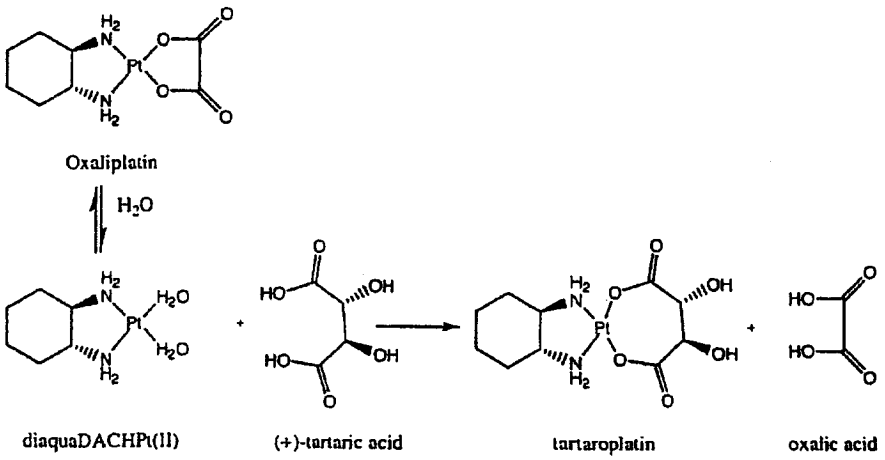

Figure 8. Illustration of Tartaroplatin Formation

Appendix A- Study Protocol

sanofi aventis

Because health matters

Industrial Analytical Development - Great Valley	
Analytical Study Protocol	Doc # IAD-060530-ELX

Analytical Assessment of Oxaliplatin Solutions Containing Tartaric Acid

(Patent Application # WO 2005/020980 A1)

<Part or all of the information in this document may be unpublished material and should be treated as the confidential property of sanofi-aventis. The use of such information or material must be restricted to the recipient from the agreed purpose and must not be disclosed to any unauthorized persons, in any form, including publications and presentations, without the written consent of sanofi-aventis. >

Analytical Assessment of Oxaliplatin Solutions Containing Tartaric Acid

(Patent Application # WO 2005/020980 A1)

1. Study Objective

The purpose of this protocol is to assess the formation of tartaroplatin through ligand exchange of oxalic acid and tartaric acid in Oxaliplatin solutions containing tartaric acid.

2. Study Outline

This study will investigate Oxaliplatin solutions containing (+)-tartaric acid. Lab scale tartaric acid containing Oxaliplatin solutions will be prepared and tested according to the according to patent application number 2005/020980 A1 titled "Acid Containing Oxaliplatin Formulation". Three tartaric acid concentrations, 0.2, 3.0, and 6.7 mM, will be assessed.

3. Material and Reference Standard

3.1 Oxaliplatin reference standard: manufactured by Tanaka will be obtained by sanofi-aventis

3.2 Oxaliplatin drug substance: manufactured by Tanaka will be obtained by sanofi-aventis

3.3 (+)-tartaric acid: Sigma Aldrich, A.C.S. reagent, ≥ 99.5%

3.4 Tartaroplatin reference solution:

This solution will serve as a retention time marker for tartaroplatin peak generated in this study.

Molecular Profiles, UK, will provide the tartaroplatin reference solution with assured and documented purity and stability (minimum of 85% purity and 4-5 hours stability at 4°C).

Molecular Profiles will provide a characterization report, which will be reviewed and approved by professor Farrell. This document will serve as a reference for confirmation of the tartaroplatin generated by ligand exchange in this study.

4. Formulation Details

Lab scale Oxaliplatin solutions containing 0, 0.2, 3.0, and 0.7 mM of tartaric acid at target pH 4.0 and 5.0 will be prepared. Detailed composition of the solutions are listed below:

Oxaliplatin concentration: 5 mg/mL
Water: Water For Injection (WFI)
Tartaric acid concentrations: 0.0, 0.2, 3.0, and 0.7 mM, equivalent to 0, 0.03, 0.45, and 1.0 mg/mL of tartaric acid.
pH: 4.0 and 5.0 (pH of the 0.0 mM will not be adjusted)

The following example provides the steps for preparation of oxaliplatin solution containing 0.2 mM tartaric acid at pH 4.0.

As per the reference patent application, one gram of Oxaliplatin will be dissolved in 200 mL of water at 50-55°C to a target concentration of 5 mg/mL. Total of 120 µL of tartaric acid solution (5% w/v) will be added to the Oxaliplatin solution to achieve target tartaric acid concentration of 0.2 mM. The pH of the solution will be adjusted to 4.0 with a range of 3.7 to 4.3 using sodium hydroxide solution.

Five additional oxaliplatin solutions containing tartaric acid will be prepared using the above example. The pH will be adjusted to the target pH of 4.0 or 5.0 according to the formula composition listed in the table below. The pH range for the target of 4.0 will be 3.7 to 4.3, and the target pH of 5.0 will be 4.7 to 5.3. The analytical control is the unbuffered Oxaliplatin solution at 5 mg/mL concentration.

The following table provides details for the preparation of the oxaliplatin solutions containing 0.2, 3.0, and 6.7 mM of (+)-tartaric acid.

Tartaric acid Concentration	Control No tartaric acid (0.0 mM)	I (0.2 mM)		II (3.0 mM)		III (6.7 mM)	
Formulation ID	Control	I4.0	I5.0	II4.0	II5.0	III4.0	III5.0
Oxaliplatin (mg)	1000	1000	1000	1000	1000	1000	1000
Tartaric Acid (mg)	0	6	6	90	90	200	200
Tartaric Acid 5% w/v (µL)	0	120	120	1800	1800	4000	4000
WFI qs (mL)	200	200	200	200	200	200	200
Target pH	Not adjusted	4.0	4.0	5.0	5.0	4.0	5.0

Note: molecular weight of tartaric acid = 150.09

I: formulation containing tartaric acid at 0.2 mM
II: formulation containing tartaric acid at 3.0 mM
III: formulation containing tartaric acid at 6.7 mM

5. Stability Testing

The solutions prepared above will be stored at ambient conditions. Tartaroplatin content will be monitored at the initial, 1, 3, 5 and 7 days.

6. Analytical Tests

Tartaroplatin content will be monitored at each time point.

7. Analytical Procedures for Tartaroplatin

The following chromatographic conditions were received and will be used in this study. This is the same as the procedure used by Molecular Profiles. This procedure has not been validated due to the lack of tartaroplatin reference standard availability.

Mobile phase: A : B = 985 : 15 (v/v)
A = water, pH adjust to 2.0 with phosphoric acid
B = methanol

HPLC column: Hypersil BDS, C18, 5µm, 250 mm by 4.6 mm
Flow rate: 1.2 mL/min
Injection volume: 10 µL
UV detection: 210 nm
Column temperature: 10°C
Autosampler temperature: 4°C

8. Tartaroplatin Concentration Calculation

The tartaroplatin levels throughout the study will be monitored and reported as %area of the total HPLC chromatographic area.

9. Summary of Results

A final analytical report will be provided upon completion of the study.