

March 6, 2000

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E. EDWARD KAVANAUGH
PRESIDENT

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Division of OTC Drug Products (HFD-560)
Office of Drug Evaluation V
Center for Drug Evaluation and Research
Food and Drug Administration
9201 Corporate Boulevard
Rockville, Maryland 20850

'00 MAR -7 19:45

Re: Final Regulation for Sunscreen Drug Products (Docket No. 78N-0038)

Dear Dr. Ganley:

This submission is made in response to the Food and Drug Administration's request from the October 26, 1999 Sunscreen Working Group Meeting and industry's commitment to provide the methods validation material on the two sunscreen control standards. Enclosed please find the method validation package for the HPLC assay for the SPF 4 and SPF 15 standard lotions.

Under separate cover, we are providing Dr. Wilson DeCamp three review copies and a desk copy addressed to his attention. We look forward to continued discussions with the Agency to resolve the technical issues associated with this rulemaking.

Respectfully submitted,

Elizabeth H. Anderson

Elizabeth H. Anderson
Assistant General Counsel

attachment

cc: Dr. Wilson DeCamp (with attachment)
Dockets Management Branch (HFA-305) (with attachment)

78N-0038

Sup 29

CTFA SPF 15 STANDARD LOTION

Assay Method
CTFA SPF 15 Standard Lotion

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CTFA SPF-15 STANDARD LOTION
Specifications

TEST	SPECIFICATION
Benzyl Alcohol	Theoretical: 0.500% w/w Limits: 0.400 to 0.600% w/w
Methylparaben	Theoretical: 0.300% w/w Limits: 0.240 to 0.360% w/w
Propylparaben	Theoretical: 0.100% w/w Limits: 0.080 to 0.120% w/w
Spectrasorb UV-9 (Oxybenzone)	Theoretical: 3.00% w/w Limits: 2.79 to 3.21% w/w
Escalol 507	Theoretical: 7.00% w/w Limits: 6.51 to 7.49% w/w

CTFA SPF 15 Standard Lotion

<u>Description</u>	<u>% w/w</u>
Benzyl Alcohol, NF	0.5000
Cocoa Butter	2.0000
Escalol 507 (Padimate O)	7.0000
Glyceryl Monostearate	3.0000
Lanolin, USP	4.5000
Methylparaben, NF	0.3000
Triethanolamine, NF	1.0000
Propylparaben, NF	0.1000
Sorbitol Solution, 70% USP	5.0000
Stearic Acid, NF	2.0000
Oxybenzone	3.0000
Water	71.6000

Sunscreen SPF 15 - Oxybenzone and Padimate O
Assay (% w/w)

A. Reagents:

1. Acetic Acid, glacial, ACS grade
2. Isopropanol, HPLC grade
3. Methanol, HPLC grade
4. Oxybenzone, Reference Standard
5. Padimate O, Reference Standard

B. Instrumentation:

Equilibrate a suitable liquid chromatograph to the following or equivalent conditions:

Column :	Ultrasphere ODS 250 x 4.6 mm (5 μ)
Mobile Phase :	85:15:0.5 Methanol:Water:Acetic Acid
Flow Rate :	1.5 mL/min.
Temperature :	Ambient
Detector :	UV Spectrophotometer @ 308 nm
Attenuation :	As needed
Injection Amount :	10 μ L

C. Mobile Phase Preparation:

Mix 850 mL methanol, 150 mL water and 5.0 mL glacial acetic acid.

D. Standard Preparation:

1. Accurately weigh about 0.50 g of Oxybenzone, Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
2. Accurately weigh about 0.50 g of Padimate O, Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
3. Accurately pipet 3.0 mL of the Oxybenzone stock solution (C.1.) and 7.0 mL of the Padimate O stock solution (C.2.) into a 100-mL volumetric flask. Dilute to volume with isopropanol and mix well. This is your Standard Preparation.

E. Sample Preparation:

1. Accurately weigh approximately 1.0 g of sample into a 50-mL volumetric flask.
2. Add approximately 30 mL of isopropanol and heat with swirling until the sample is evenly dispersed.
3. Cool to room temperature and dilute to volume with isopropanol. Mix well.

Sunscreen SPF 15 – Oxybenzone and Padimate O
Assay (% w/w)

4. Pipet 5.0 mL of the sample solution (D.3.) into a 50-mL volumetric flask and dilute to volume with isopropanol. Mix well.

F. System Suitability:

An HPLC equilibrated to the above conditions would be considered suitable. This system would insure that three replicate injections of the Standard Preparation would yield a relative standard deviation of not more than 2.0% calculated on peak areas for Oxybenzone and Padimate O. The system would also ensure a calculated resolution between the Oxybenzone and Padimate O peaks of not less than 3.0.

G. Analysis:

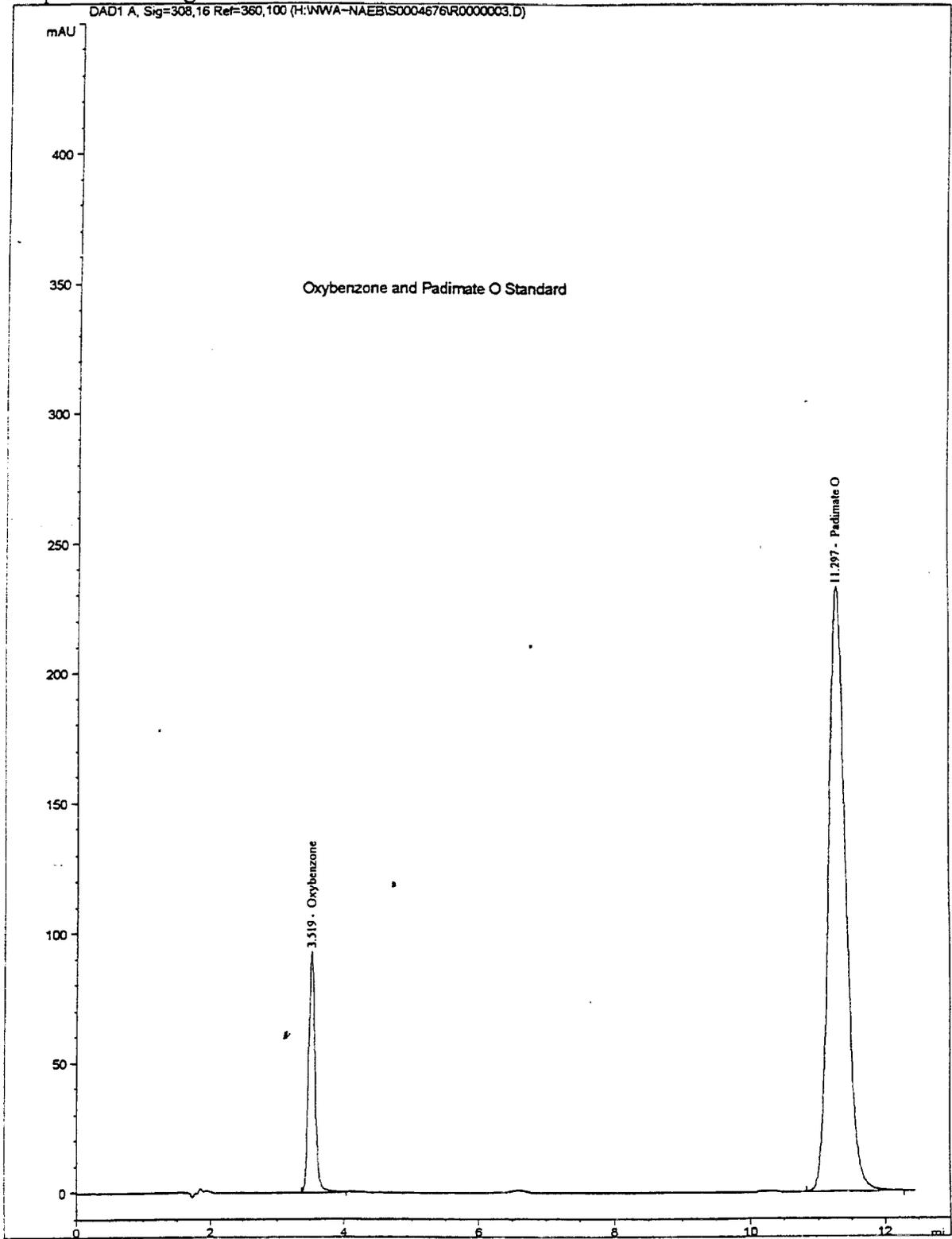
1. Inject 10 μ L of the Standard Preparation in triplicate collecting data for about 15 minutes or until the Padimate O peak has completely eluted. Determine if the system meets the suitability criteria as established above. Elution order: (1) Oxybenzone (2) Padimate O.
2. Similarly inject 10 μ L of each Sample Preparation.
3. Calculate the percent of each sunscreen in the sample as follows:

$$\frac{(\text{Smp. Oxybenzone Peak Area})(\text{Std. Oxybenzone Wt. g})(6)}{(\text{Std. Oxybenzone Peak Area})(\text{Smp. Wt. g})} = \text{Oxybenzone \% (w / w)}$$

$$\frac{(\text{Smp. Padimate O Peak Area})(\text{Std. Padimate O Wt. g})(14)}{(\text{Std. Padimate O Peak Area})(\text{Smp. Wt. g})} = \text{Padimate O \% (w / w)}$$

Sunscreen SPF 15 - Oxybenzone and Padimate O
Assay (% w/w)

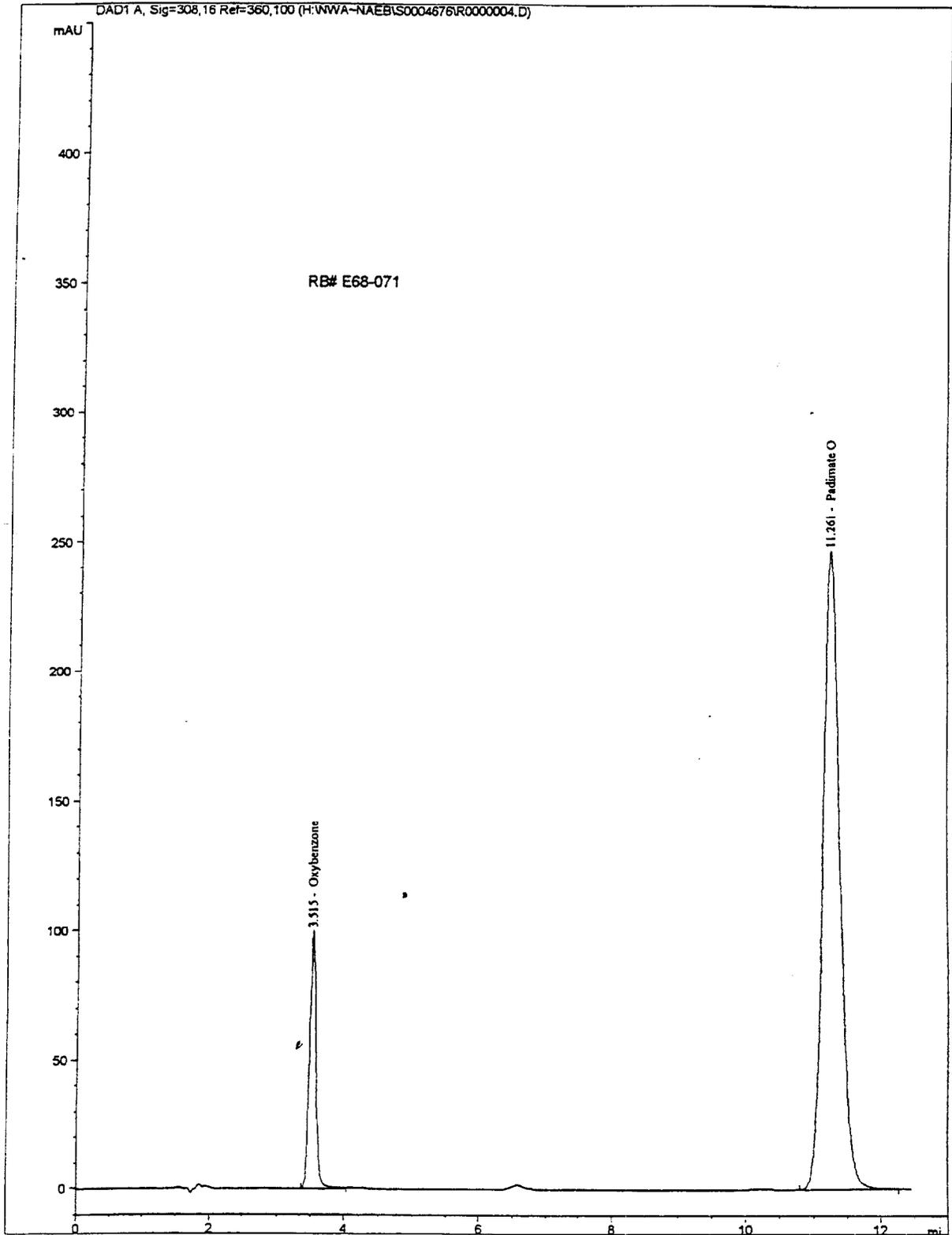
Example Chromatograms:



Typical Standard Chromatogram

005

Sunscreen SPF 15 - Oxybenzone and Padimate O
Assay (% w/w)



Typical Sample Chromatogram

006

SCHERING-PLOUGH HEALTHCARE PRODUCTS

RESEARCH AND DEVELOPMENT

ANALYTICAL RESEARCH

ANALYTICAL METHOD VALIDATION REPORT

Validation Number: 990034

RB# E68-071, CTFA SPF-15 STANDARD LOTION

Assay of Oxybenzone and Padimate O

December 10, 1999

007

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I. INTRODUCTION

Purpose: This document describes the experiments performed to validate the assay procedure used to determine the level of Oxybenzone and Padimate O in the following formula.

RB# E68-071, CTFA SPF-15 Standard Lotion

Formulation: The formula contains the following sunscreen actives in a lotion matrix:

Oxybenzone	3.00% w/w
Padimate O	7.00% w/w.

This formula also contains benzyl alcohol, cocoa butter, glyceryl monostearate, lanolin, methylparaben, triethanolamine, propylparaben, sorbitol solution, 70%, stearic acid and water.

The sunscreens detected by the analytical method are Oxybenzone and Padimate O. The analytical method may be found in ATTACHMENT 1.

The chemical name for Oxybenzone is (2-Hydroxy-4-methoxyphenyl)phenylmethanone. The empirical formula is $C_{14}H_{12}O_3$ and the structure is in Figure 1.

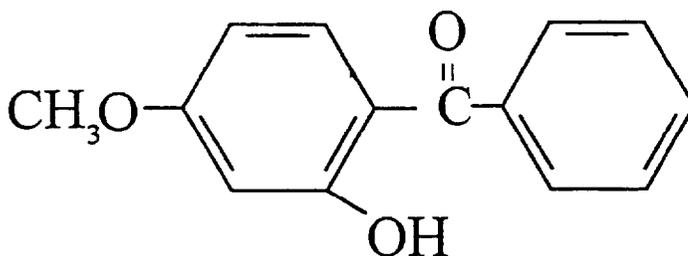


Figure 1. Oxybenzone

The chemical name for Padimate O, (Escalol 507) is octyl dimethyl paba. The empirical formula is $C_{17}H_{27}NO_2$ and the structure is in Figure 2.

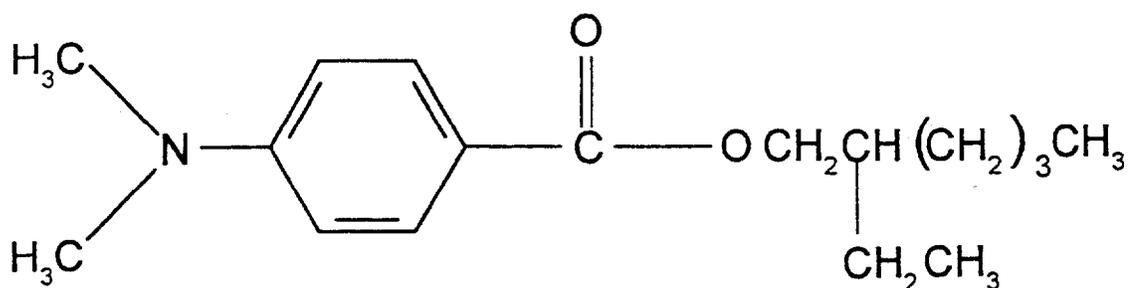


Figure 2. Padimate O

Method Information: The proposed analytical method for the assay of Oxybenzone and Padimate O in this formula uses high performance liquid chromatography (HPLC) with a C₁₈ reverse-phase column to achieve separation. Detection of the actives is by UV absorbance at a wavelength of 308nm.

A copy of the analytical method for the assay of Oxybenzone and Padimate O in this product is presented in ATTACHMENT 1.

This report describes the experiments performed and data generated to validate this analytical method. It demonstrates the suitability of the method to quantitate Oxybenzone and Padimate O in this product.

The following experiments were performed for the validation of the analytical method for the assay of Oxybenzone and Padimate O in CTFA SPF-15 Standard Lotion:

- A. Evaluation of Linearity and Working Concentration Range of the Standard.
- B. Evaluation of Accuracy and Recovery from Spiked Placebos.
- C. Evaluation of System Precision.
- D. Evaluation of Repeatability.
- E. Evaluation of Reproducibility.
- F. Evaluation of Standard/Sample Stability.
- G. Evaluation of Method Robustness.
- H. Evaluation of Specificity.

II EXPERIMENTAL

The samples used for this validation included:

RB# E68-071, Lot# P58010, CTFA SPF-15 Standard Lotion
RB# P58-014, CTFA SPF-15 Standard Lotion, without Oxybenzone
RB# P58-016, CTFA SPF-15 Standard Lotion, without Padimate O.

The reference materials used in this validation were approved standards. All solvents used were HPLC grade.

All equipment used in this validation was in calibration as per appropriate standard operating procedures.

Assay testing was performed in accordance with the analytical method in ATTACHMENT 1.

III. RESULTS

A. Evaluation of Linearity and Working Concentration Range of the Standard

Linearity is defined as the ability of an analytical method to detect a proportional response to increasing or decreasing analyte concentration. The range of an analytical method is the interval between upper and lower levels of the analyte (including these levels) that have been determined with a suitable level of precision, accuracy and linearity. For true linear response, the ratio of system response to concentration (response factor) will remain constant as concentration changes.

Standard solutions were prepared at 50%, 80%, 100%, 120% and 150% of the theoretical content of oxybenzone and padimate O in the working standard. After system suitability was established, each level was injected in triplicate.

The response factors (RF) at each level were calculated using the following equation:

$$\text{Response Factor} = \frac{\text{System Response}}{\text{Concentration}}$$

The tabulated results are shown in Tables 1 and 2.

The data was analyzed using linear regression analysis with the known concentration (mg/ml) as the independent (X) value and the system response (peak area) as the dependent (Y) value. The linear response (Figures 3 and 5) is demonstrated by a high coefficient of determination.

The response factors at each level were averaged. The data was plotted with the standard concentration (expressed as % Theory Added) and the mean response factor as the Y-value. Parallel lines were drawn at 2% above and below the response factor at 100% of the theoretical working concentration. This response plot assesses the concentration range where the response factors are consistent within experimental variability. This is defined as the working range.

Figure 4 shows that the average response factors for oxybenzone are within $\pm 2\%$ of the average response factor at 100% for a range of 50 to 150%.

Figure 6 shows that the average response factors for padimate O are within $\pm 2\%$ of the average response factor at 100% for a range of 50 to 150%.

Table 1. Oxybenzone Linearity

% Theory Added	Concentration	Response	Response Factor	AVG RF	%RSD
50.38	0.03023	314.27	10396	10391	0.06
	0.03023	313.92	10384		
	0.03023	314.13	10391		
80.61	0.04837	499.07	10318	10313	0.07
	0.04837	498.45	10305		
	0.04837	498.99	10316		
100.77	0.06046	627.01	10371	10362	0.09
	0.06046	625.90	10352		
	0.06046	626.52	10363		
120.92	0.07255	749.28	10328	10327	0.02
	0.07255	749.39	10329		
	0.07255	749.05	10325		
151.15	0.09069	936.55	10327	10320	0.07
	0.09069	935.83	10319		
	0.09069	935.33	10313		

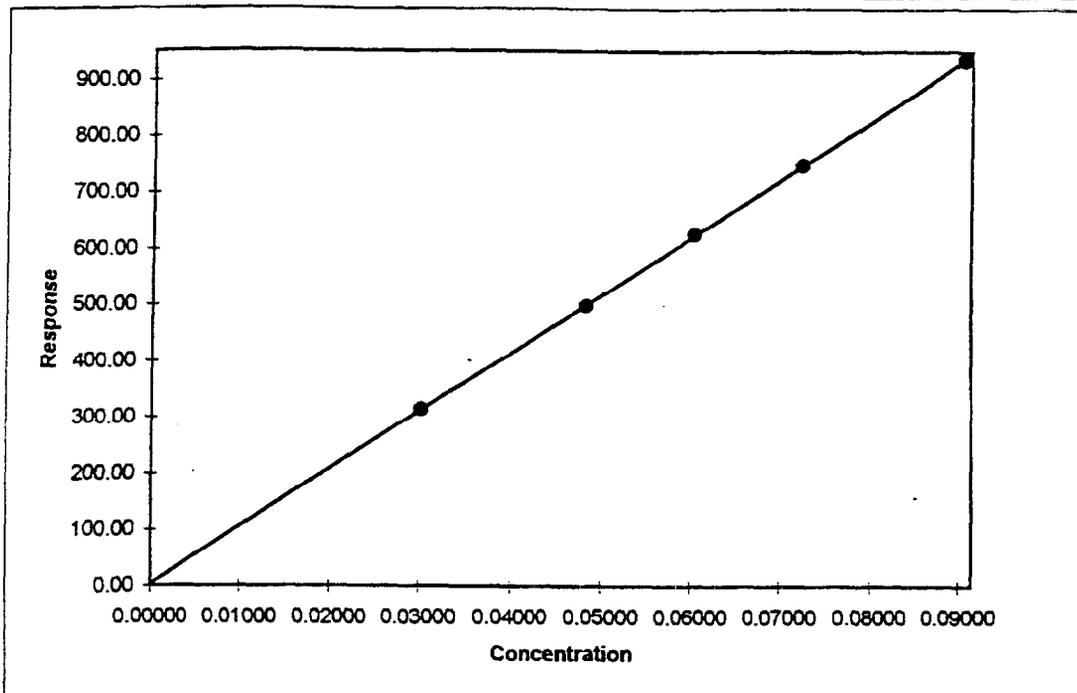


Figure 3. Oxybenzone Linearity

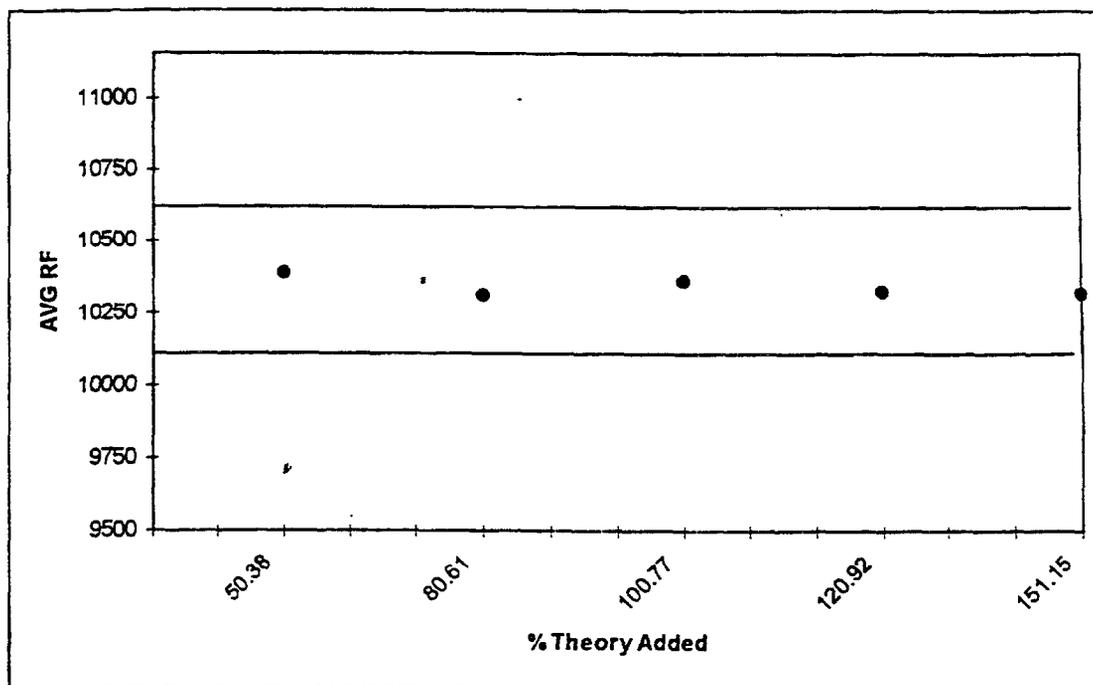


Figure 4. Oxybenzone Response Factors

Table 2. Padimate O Linearity

% Theory Added	Concentration	Response	Response Factor	AVG RF	%RSD
50.26	0.070358	2055.4	29213	29198	0.05
	0.070358	2053.4	29185		
	0.070358	2054.2	29196		
80.41	0.112573	3256.2	28925	28911	0.05
	0.112573	3253.2	28899		
	0.112573	3254.5	28910		
100.51	0.140716	4091.1	29073	29059	0.04
	0.140716	4087.7	29049		
	0.140716	4088.4	29054		
120.61	0.168859	4889.3	28955	28952	0.01
	0.168859	4888.4	28950		
	0.168859	4888.5	28950		
150.77	0.211074	6107.6	28936	28921	0.05
	0.211074	6104.7	28922		
	0.211074	6101.2	28906		

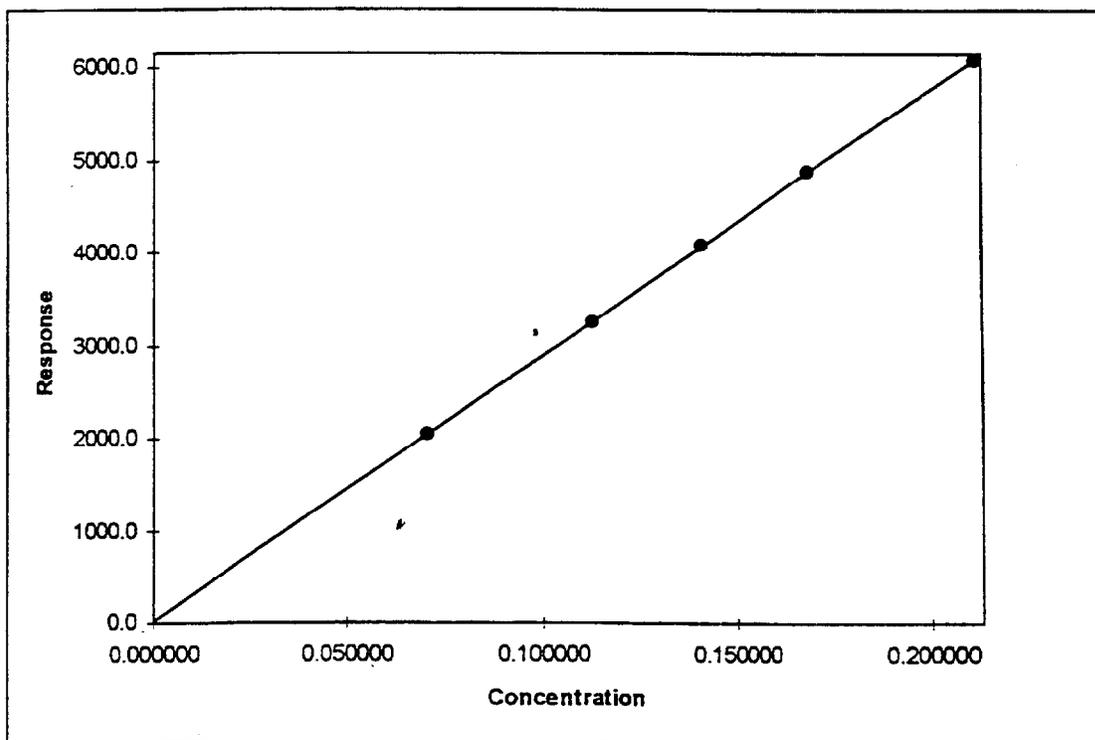


Figure 5. Padimate O Linearity

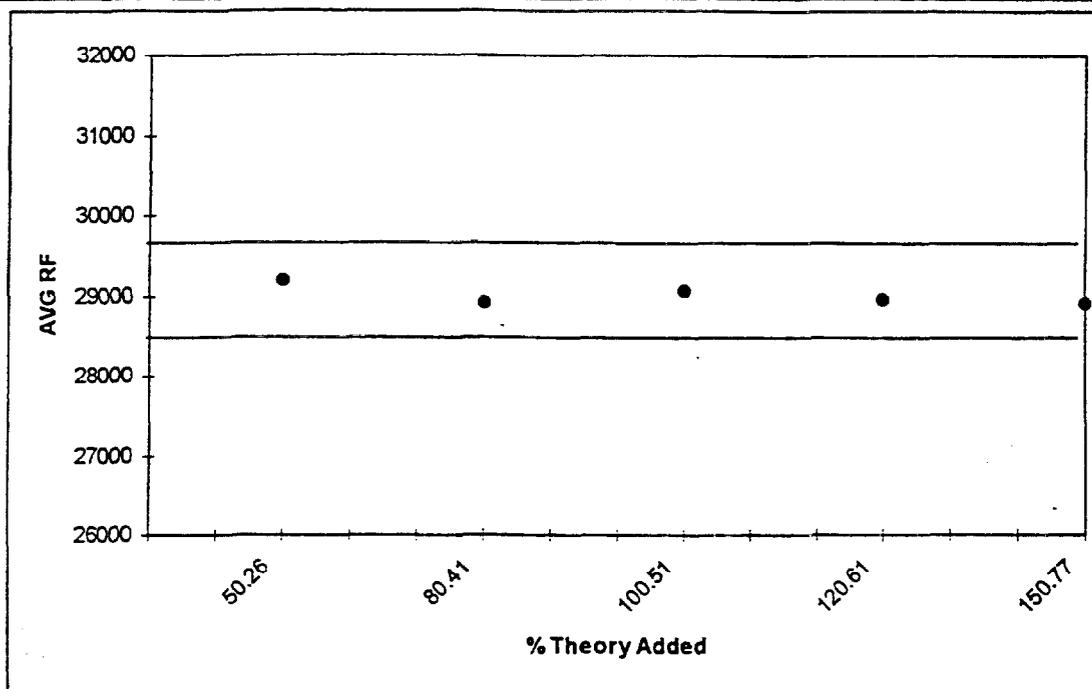


Figure 6. Padimate O Response Factors

Acceptance Criteria:

- Coefficient of Determination (r^2) ≥ 0.99 .
- %RSD for the average Response Factor at each level ≤ 2.0 .
- The average Response Factor at each level is within $\pm 2.0\%$ of the average Response Factor at the 100% level.

Conclusion:

Linear regression analysis was performed on the oxybenzone data using the concentration (mg/ml) as the independent (x) variable and the system response (peak area) as the dependent (y) variable. The calculated regression equation is $Y = 10294.3 X + 2.5210$, with a coefficient of determination (r^2) of 1.0000.

The %RSD of the oxybenzone response factors at each level is $\leq 2.0\%$. The Response Factor plot (Figure 4) shows that for a range of 50 to 150%, the average response factor is within $\pm 2.0\%$ of the average Response Factor at 100%.

Linear regression analysis was performed on the padimate O data using the concentration (mg/ml) as the independent (x) variable and the system response (peak area) as the dependent (y) variable. The calculated regression equation is $Y = 28817 X + 23.2466$, with a coefficient of determination (r^2) of 1.0000.

The %RSD of the padimate O response factors at each level is $\leq 2.0\%$. The Response Factor plot (Figure 6) shows that for a range of 50 to 150%, the average response factor is within $\pm 2.0\%$ of the average Response Factor at 100%.

The analytical method meets the acceptance criteria for linearity and range.

B. Evaluation of Accuracy and Recovery from Spiked Placebos

Accuracy is defined as the ability of the sample preparation to extract the analyte from the sample matrix to which known amounts of drug substance have been added. Stock standards were prepared for each analyte as spiking solutions. One placebo stock was prepared. Four placebo blend dilutions were spiked with 0, 50, 100 and 150% of the theoretical amount of Oxybenzone. The placebo blend preparations were repeated for Padimate O. These samples were analyzed according to the analytical procedure in ATTACHMENT 1. Each sample preparation was injected in triplicate. Tables 3 and 4 contain the tabulated results showing % Theory Added (spike level), % Theory Found and % Recovery at each level for each analyte. Figures 7 and 8 are graphical representations of the linear regression.

Table 3. Recovery of Oxybenzone from Spiked Placebos, RB# P58-014

SAMPLE	% THEORY ADDED	% THEORY FOUND	% RECOVERY
0	0.00	0.00	0.00
0	0.00	0.00	0.00
0	0.00	0.00	0.00
50	50.34	50.23	99.78
50	50.34	50.24	99.80
50	50.34	50.21	99.74
100	100.67	100.99	100.32
100	100.67	100.73	100.06
100	100.67	100.78	100.11
150	151.01	151.56	100.36
150	151.01	151.26	100.17
150	151.01	151.29	100.19

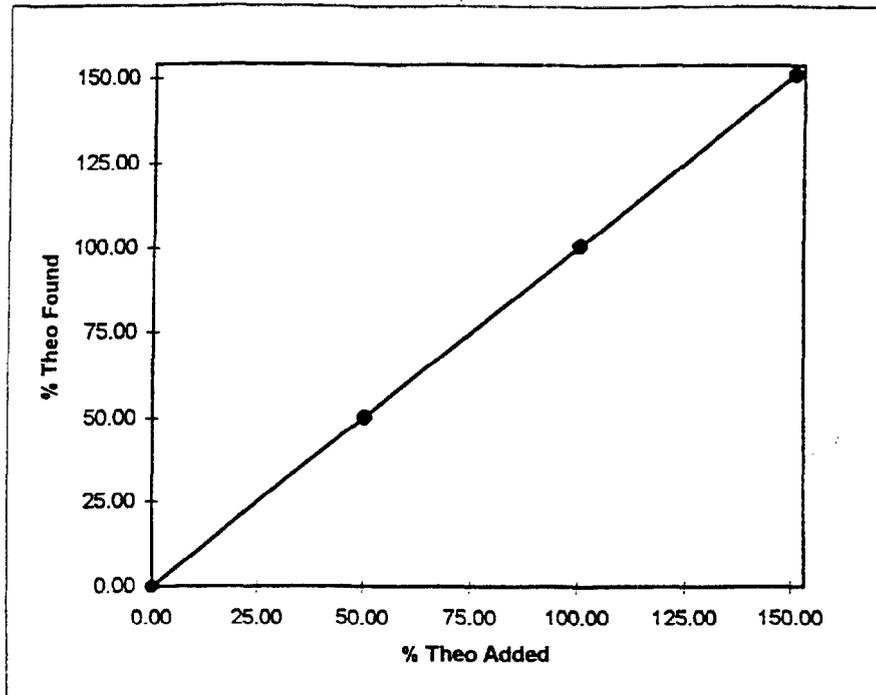


Figure 7. Oxybenzone Recovery from Spiked Placebos, RB# P58-014

Table 4. Recovery of Padimate O from Spiked Placebos, RB# P58-016

SAMPLE	% THEORY ADDED	% THEORY FOUND	% RECOVERY
0	0.00	0.00	0.00
0	0.00	0.00	0.00
0	0.00	0.00	0.00
50	51.33	51.11	99.57
50	51.33	51.06	99.47
50	51.33	51.03	99.42
100	102.66	102.51	99.85
100	102.66	102.52	99.86
100	102.66	102.67	100.01
150	153.99	153.44	99.64
150	153.99	153.49	99.68
150	153.99	153.07	99.40

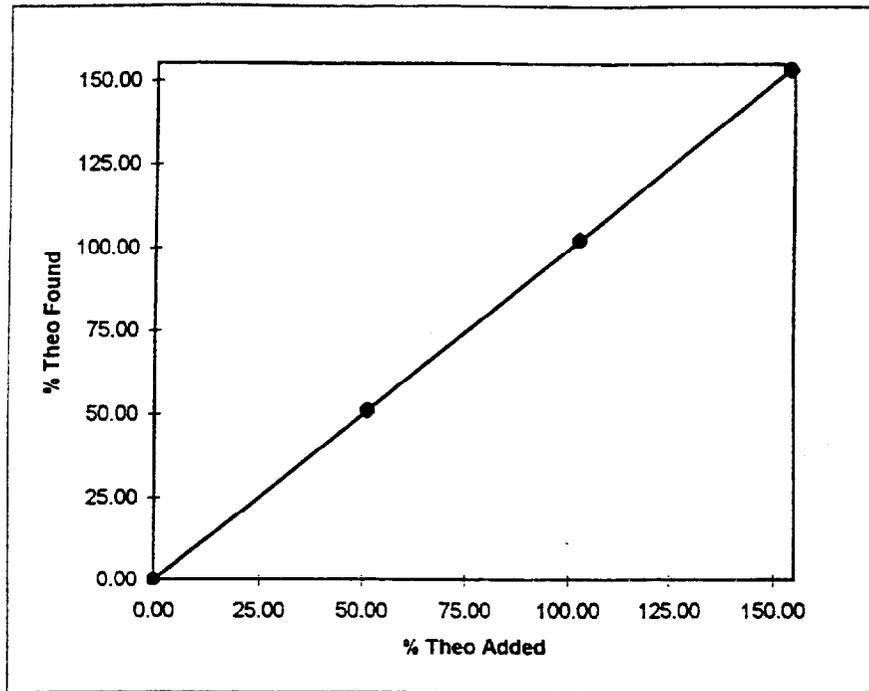


Figure 8. Recovery of Padimate O from Spiked Placebos, RB# P58-016

Acceptance Criteria:

For each analyte:

- The coefficient of determination (r^2) is ≥ 0.99 .
- At the 95% confidence limits, the slope is 1.0.
- At the 95% confidence limits, the intercept is 0.0.
- The average recovery at each level is 98-102%.
- The percent error due to the intercept at 80 and 120% of theoretical is $-2\% \leq x \leq 2\%$.

Conclusion:

A linear regression was performed on the data for each analyte using % Theory Added as the independent (x) variable and % Theory Found as the dependent (y) variable.

The linear regression equation for Oxybenzone is

$$Y = 1.0027 X - 0.1010$$

with a coefficient of determination (r^2) of 1.0000. The percent error due to the intercept at 80% is -0.0252 and at 120% is 0.0168 of theoretical working concentration for the analyte. The average recovery at each level is within the range of 98-102%.

The linear regression equations for Padimate O is

$$Y = 0.9965 X + 0.0167$$

with a coefficient of determination (r^2) of 1.0000. The percent error due to the intercept at 80% is 0.0042 and at 120% is -0.0028 of theoretical working concentration for the analyte. The average recovery at each level is within the range of 98-102%.

The analytical method meets the acceptance criteria for accuracy.

C. Evaluation of System Precision:

System precision is established by calculating the %RSD of multiple standard injections performed throughout an analysis. This not only confirms that acceptable precision is obtained initially for the system suitability test, but also that standards repeated throughout an analysis continue to meet system suitability criteria.

During the Oxybenzone linearity study six standards were injected. The average, standard deviation, and %RSD of the response factors for each analyte were calculated. Table 5 contains these results

Table 5. System Precision

Results	Oxybenzone	Padimate O
Peak Areas	628.12	4132.4
Std Dev	1.1267	8.6523
%RSD	0.18	0.21

Acceptance Criteria:

- The %RSD of the response factors for each analyte is ≤ 2.0 .

Conclusion:

The system precision data for each analyte meets the acceptance criteria.

D. Evaluation of Method Precision (Repeatability)

Method precision (Repeatability) was demonstrated by analyzing 6 sample preparations and determining each analyte content as described in the analytical method in ATTACHMENT 1. The average and % RSD were calculated for the assay results for each analyte. Table 6 contains the data.

Table 6. Oxybenzone and Padimate O Repeatability

FORMULA RB# E68-071	OXYBENZONE % w/w	PADIMATE O % w/w
1	3.01	6.96
2	3.02	6.97
3	3.02	6.99
4	3.03	6.99
5	3.03	6.99
6	3.03	6.97
MEAN	3.02	6.98
STD DEV	0.0082	0.0133
% RSD	0.27	0.19

Acceptance Criteria:

- For each analyte, the %RSD of the assay results is $\leq 2.0\%$.

Conclusion:

For each analyte, the % RSD for the assay results is ≤ 2.0 . The analytical method meets the acceptance criteria for repeatability.

E. Evaluation of Reproducibility

Reproducibility of the method is established to ensure that different laboratories can obtain comparable results within acceptable levels of precision and accuracy. Reproducibility is determined by analyzing the assay data from two different laboratories using different analysts performing replicate sample preparations on different days. The data generated by the laboratories is subjected to statistical treatment in order to calculate the 95% confidence limits for the mean difference between laboratories.

Two replicate samples from a single batch of the product were prepared and assayed in duplicate in LAB 1 (Analytical Validations) by a single analyst on 2 days. The product was sent to LAB 2 (Analytical Stability) where a second analyst assayed them as above. Tables 7 and 8 contain the data from the reproducibility testing.

Table 7. Oxybenzone Ruggedness, RB# E68-071

LAB	DAY	SAMPLE	INJECTION	OXYBENZONE %w/w	% THEORY
1	1	1	1	3.01	100.3
			2	3.01	100.3
		2	1	3.02	100.7
			2	3.01	100.3
	2	1	1	3.06	102.0
			2	3.06	102.0
		2	1	3.05	101.7
			2	3.04	101.3
2	1	1	1	3.02	100.7
			2	3.02	100.7
		2	1	3.01	100.3
			2	3.01	100.3
	2	1	1	3.05	101.7
			2	3.05	101.7
		2	1	3.05	101.7
			2	3.05	101.7
95% Confidence limits for the mean difference between labs				± 0.227	
Difference between labs				-0.025	
%RSD for pooled results-both labs				0.682	
%RSD for results each lab				Lab 1 0.757 Lab 2 0.652	

Table 8. Padimate O Ruggedness, RB# E68-071

LAB	DAY	SAMPLE	INJECTION	PADIMATE O %w/w	% THEORY
1	1	1	1	6.94	99.14
			2	6.94	99.14
		2	1	6.97	99.57
			2	6.96	99.43
	2	1	1	6.98	99.71
			2	6.97	99.57
		2	1	6.93	99.00
			2	6.92	98.86
2	1	1	1	6.94	99.14
			2	6.93	99.00
		2	1	6.91	98.71
			2	6.91	98.71
	2	1	1	6.95	99.29
			2	6.95	99.29
		2	1	6.95	99.29
			2	6.95	99.29
95%RSD Confidence limits for the mean difference between labs				± 0.305	
Difference between labs				0.213	
%RSD for pooled results-both labs				0.297	
%RSD for results each lab				Lab 1 0.311 Lab 2 0.259	

Acceptance Criteria:

For each analyte:

- The 95% confidence limits for the mean difference between laboratories is ≤ 4.0 .
- The difference between laboratory means is $\leq 3.0\%$.
- The %RSD for the pooled results from both laboratories is ≤ 2.5 .
- The %RSD for the results from each laboratory is $\leq 2.0\%$.

Conclusion:

The analytical method meets the acceptance criteria for reproducibility.

F. Evaluation of Standard/Sample Stability

The stability of the assay standard and sample preparations was determined. A standard and sample were prepared and assayed for each analyte as described in the method in ATTACHMENT 1. These solutions remained in a cabinet and at room temperature for the duration of the experiment. Tables 9 through 12 contain the results of this experiment.

The chromatograms were reviewed for indications of any degradation and stability of the standard and sample solutions.

Table 9. Stability of Standard

OXYBENZONE			
	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	10387.8	10346.7	10356.6
% DIFFERENCE	N/A	0.40	0.30

Table 10. Stability of Standard

PADIMATE O			
	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	29174.0	29072.4	29086.5
% DIFFERENCE	N/A	0.35	0.30

Table 11. Stability of Sample, RB# E68-071

OXYBENZONE			
	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	10220.9	10187.5	10192.1
% DIFFERENCE	N/A	0.33	0.28

Table 12. Stability of Sample, RB# E68-071

PADIMATE O			
	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	28423.6	28334.7	28353.5
% DIFFERENCE	N/A	0.31	0.25

Acceptance Criteria:

For each analyte:

- The % difference between response factors at the interval and those obtained at time 0 is $\leq 2.0\%$. If the % difference is $> 2.0\%$ the method should specify to prepare fresh daily.

Conclusion:

There was no indication of any analyte degradation in the standard or sample preparation over the 48 hour period. The analytical method meets the acceptance criteria for the stability of standard and sample solutions.

G. Evaluation of Method Robustness

Robustness is determined by making small but deliberate changes to method parameters and evaluating their effect on the overall analytical system. Typical liquid chromatographic condition changes include organic strength, pH, flow rate, buffer, and column temperature. Any parameter that is found to produce an undesirable effect should be identified in the method as a critical parameter and appropriate cautions included in the method.

The robustness of this method was tested by varying the flow rate, organic strength, and acid strength of the mobile phase. In addition, the column was replaced with a second column containing a different lot of packing material to demonstrate system suitability.

The variation in the system and the effect of the changes on retention time, and resolution of the oxybenzone and padimate O peaks in the sample preparation are included in this report.

The effects of changes due to flow rate are in Table 13 and Figure 9.

Table 13. Flow Rate

Method Parameters	Retention Time Oxybenzone	Retention Time Padimate O	Resolution
Flow Rate of 1.5ml/min	3.39	10.32	23.43
Flow Rate of 1.2 ml/min	4.24	12.87	24.66
Flow Rate of 1.8 ml/min	2.83	8.58	22.16

Increasing and decreasing the flow rate moved the peaks slightly. These changes in flow rate were not critical to the method.

The effects of changes due to organic/aqueous ratio are in Table 14 and Figure 10.

Table 14. Organic/Aqueous

Method Parameters	Retention Time Oxybenzone	Retention Time Padimate O	Resolution
Mobile Phase: 85:15:0.5 Methanol:H2O:Acetic Acid	3.39	10.32	23.43
Organic/Aqueous 80:20:0.5	4.32	18.41	30.58
Organic/Aqueous 90:10:0.5	2.80	6.24	16.14

Increasing the organic ratio decreased the retention times of the analytes significantly.

The effects of changes due to glacial acetic acid concentration are in Table 15 and Figure 11.

Table 15. Glacial Acetic Acid

Method Parameters	Retention Time Oxybenzone	Retention Time Padimate O	Resolution
5ml Glacial Acetic Acid	3.39	10.32	23.43
4.5ml Glacial Acetic Acid	3.32	9.77	22.57
5.5ml Glacial Acetic Acid	3.34	9.87	22.48

Increasing and decreasing the glacial acetic acid by 0.5ml did not significantly affect the chromatography.

The effects of changes due to second column containing a different lot of packing material are in Table 16 and Figure 12.

Table 16. Columns – Packing Materials

Method Parameters Column	Retention Time Oxybenzone	Retention Time Padimate O	Resolution
CO025095/8UE4203	3.39	10.32	23.43
CO025094/7UE1325	3.55	11.64	23.92

Both columns meet system suitability criteria for this method.

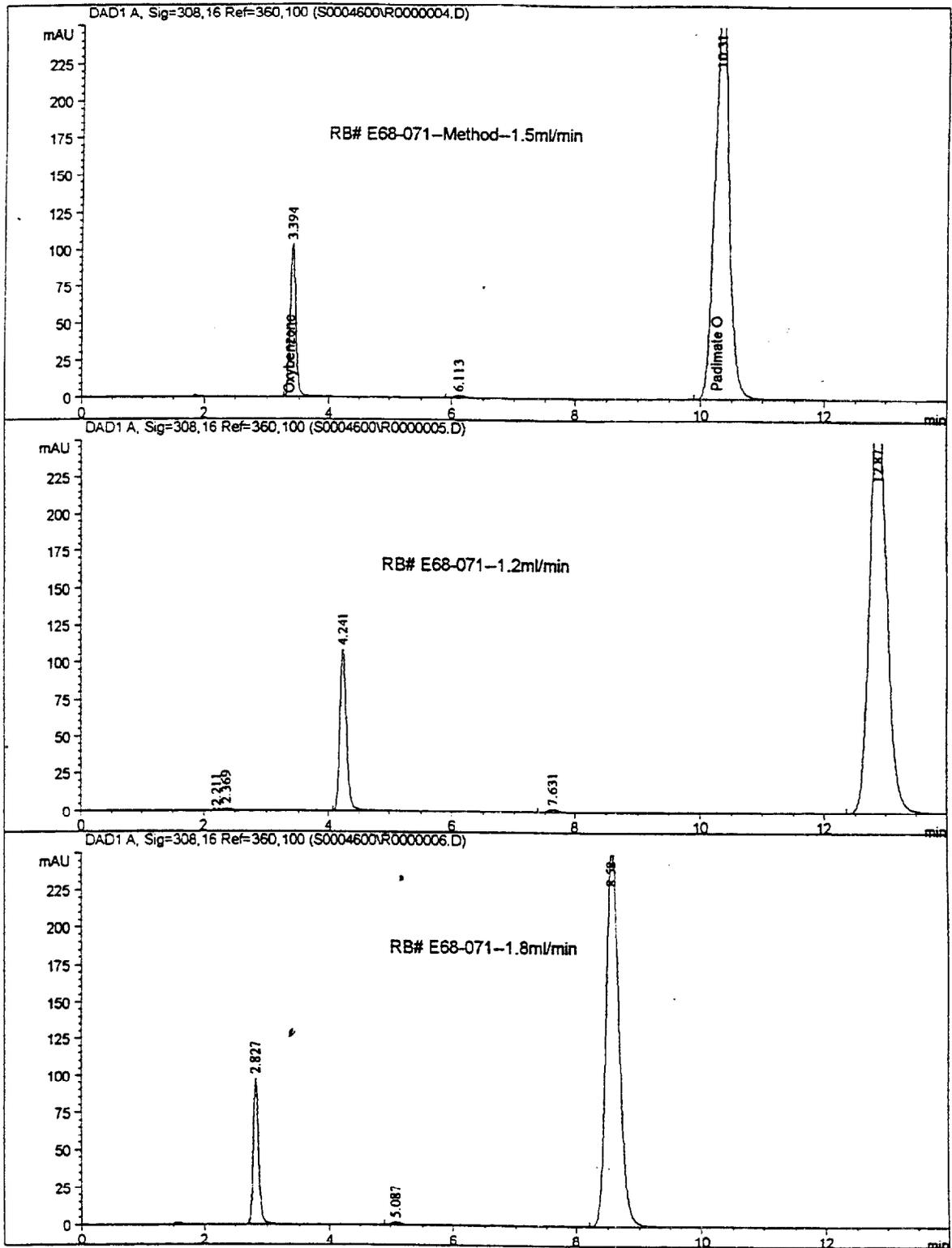


Figure 9. Effect of Flow Rate Variation

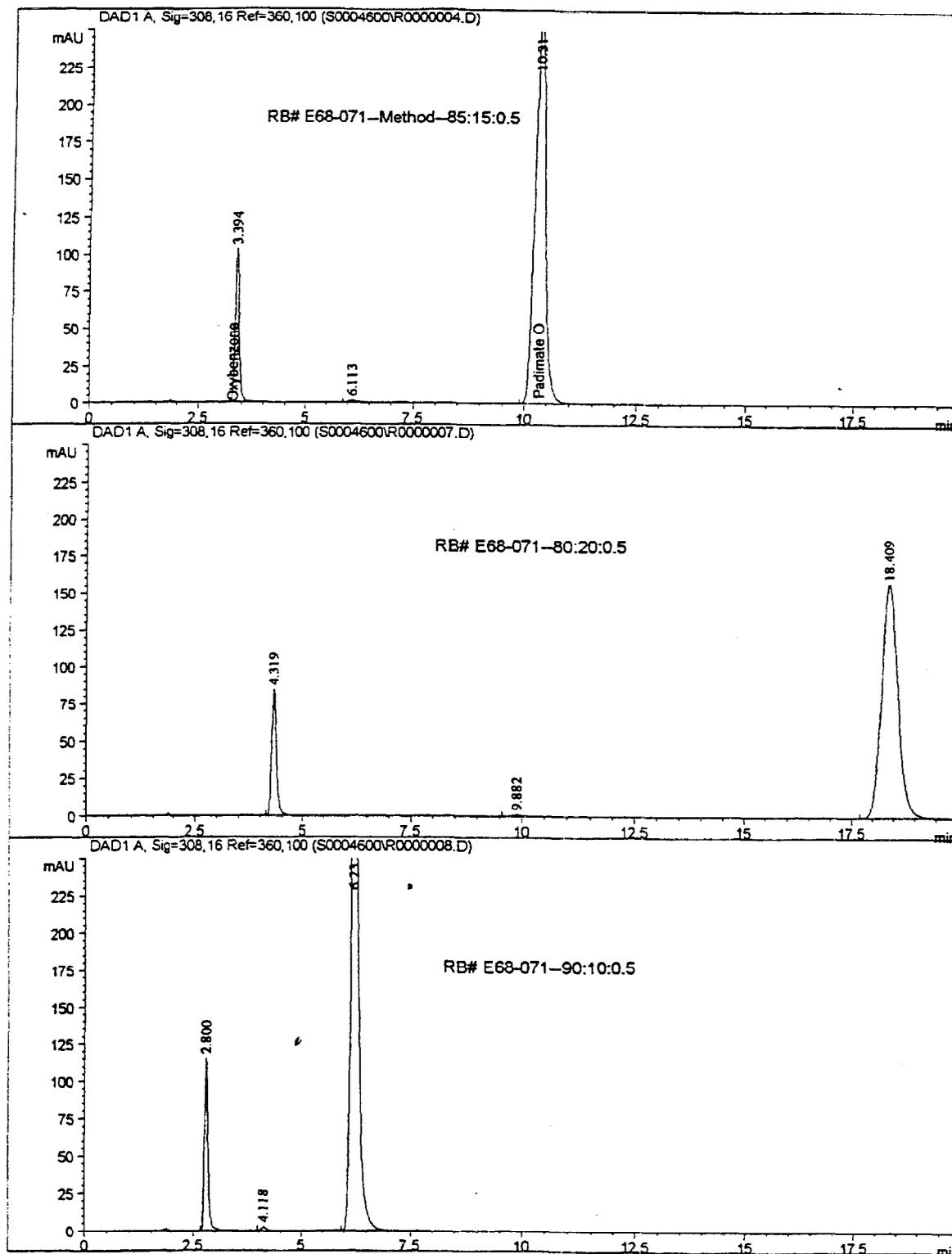


Figure 10. Effect of Organic/Aqueous Variation

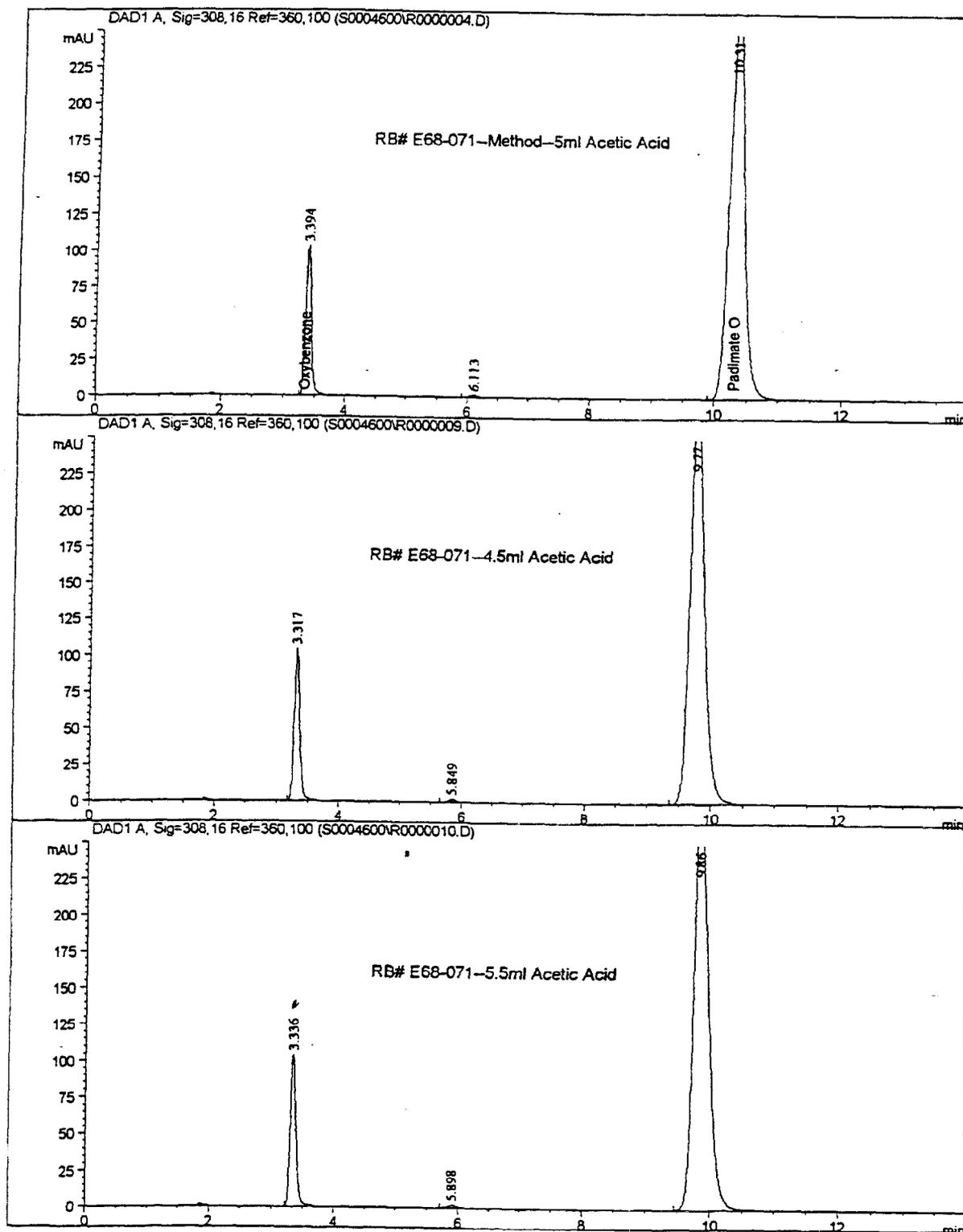


Figure 11. Effect of Glacial Acetic Acid

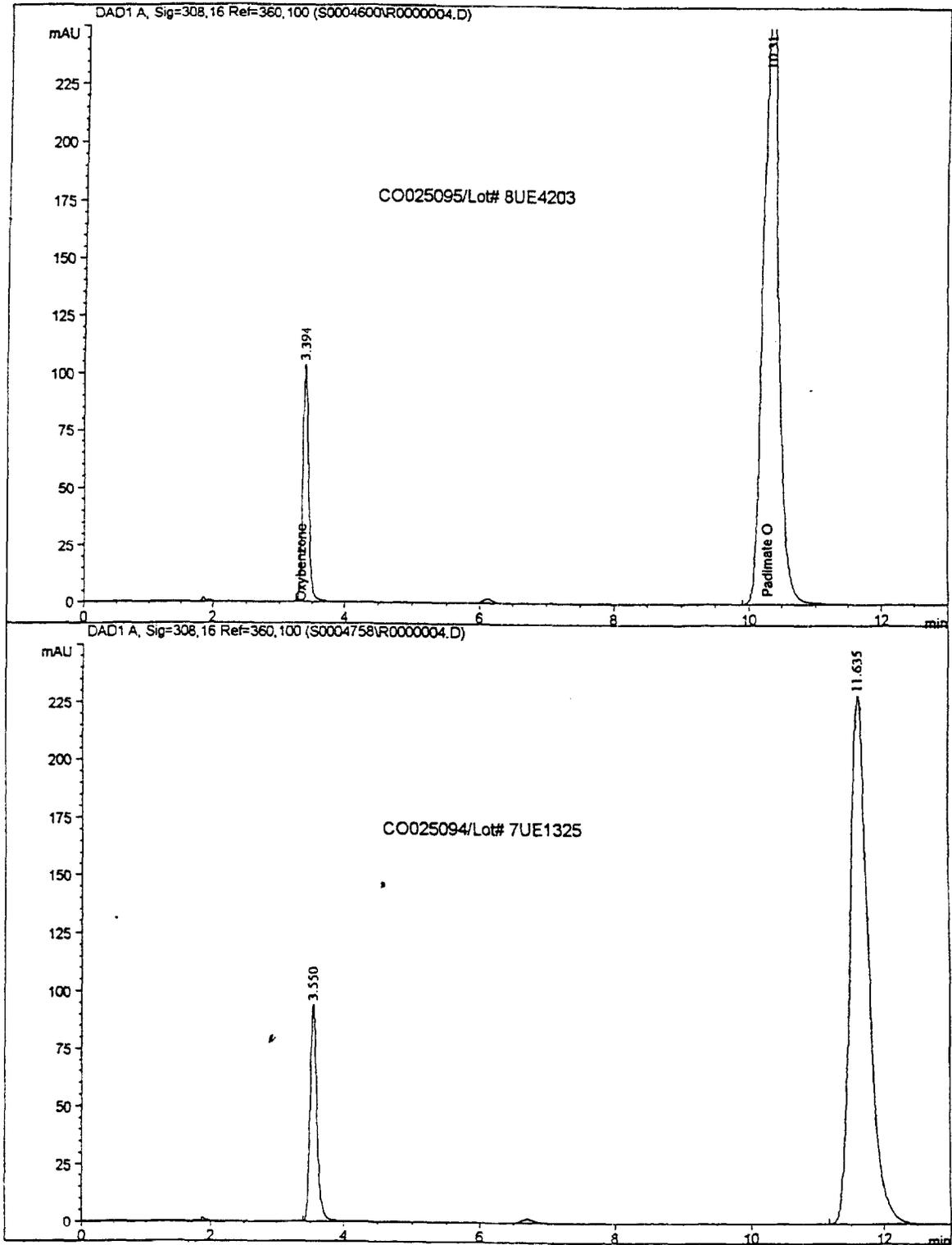


Figure 12. Effect of column containing different lot of packing material

Acceptance Criteria:

- Ensure that critical method parameters are identified in the analytical method.

Conclusion:

Variations in organic strength of the mobile phase cause the greatest changes in retention of the analytes. The analytical method meets the acceptance criteria for robustness.

H. Evaluation of Method Specificity

Specificity is defined as the ability of an analytical method to discriminate the analyte being quantitated without interference from other formula ingredients or degradation products.

To demonstrate the specificity of the analytical method, the formula and placebos were exposed to heat and light conditions. In addition, the analytes were physically and chemically stressed.

All heat stress experiments were performed at 60°C in a suitably calibrated oven for two weeks. The light stress experiments were carried out in a light cabinet calibrated to ensure a light density of 1400 foot candles (15,000 lux) for two weeks

Forced degradation of the analytes was performed as follows:

<u>TYPE</u>	<u>CONDITION</u>
Acid	0.1N HCl heated on steam bath for 1 hour.
Base	0.1N NaOH heated on steam bath for 1 hour.
Peroxide	3% H ₂ O ₂ heated gently for 1 hour.

All stressed and unstressed samples were assayed as described in the method in ATTACHMENT 1, using an instrument equipped with a photodiode array detector.

Peak purity of the oxybenzone and padimate O peaks were determined and chromatograms of the stressed and unstressed placebos were examined for interferences. Purity factors were evaluated. A numerical value of 1000 indicates a perfect match of spectra generated from the analyte peak. If this match is 990 or greater, this indicates the peak is pure. The percent recovery is also reported. Table 17 contains peak purity data.

Table 17. Purity Factors of Unstressed and Stressed Materials

Sample Information		Oxybenzone		Padimate O	
Sample Condition	Sample Amount	Purity	% Recovery	Purity	% Recovery
E68-071 Unstressed	1.0774g	999.996	100.3	999.998	99.19
E68-071 Heat	1.0057g	999.997	91.47	999.998	89.71
E68-071 Light	1.0131g	999.996	99.54	999.998	97.54
Oxybenzone RS018807 Unstressed	0.5042g	999.997	99.90	—	—
Oxybenzone Heat	102.50mg	999.997	100.1	—	—
Oxybenzone Light	102.03mg	999.997	100.1	—	—
Oxybenzone Acid	102.01mg	999.996	81.36	—	—
Oxybenzone Base	100.97mg	999.996	99.57	—	—
Oxybenzone Peroxide	102.38mg	999.997	98.95	—	—
Padimate O RS025805 Unstressed	0.5062g	—	—	999.998	99.98
Padimate O Heat	101.93mg	—	—	999.998	99.00
Padimate O Light	103.63mg	—	—	999.998	95.42
Padimate O Acid	102.86mg	—	—	999.998	102.1
Padimate O Base	101.38mg	—	—	999.998	99.07
Padimate O Peroxide	101.82mg	—	—	999.998	98.16

Figure 13 is a typical standard chromatogram. Figure 14 is a typical sample chromatogram. Figures 15 through 21 are chromatograms for stressed and unstressed materials.

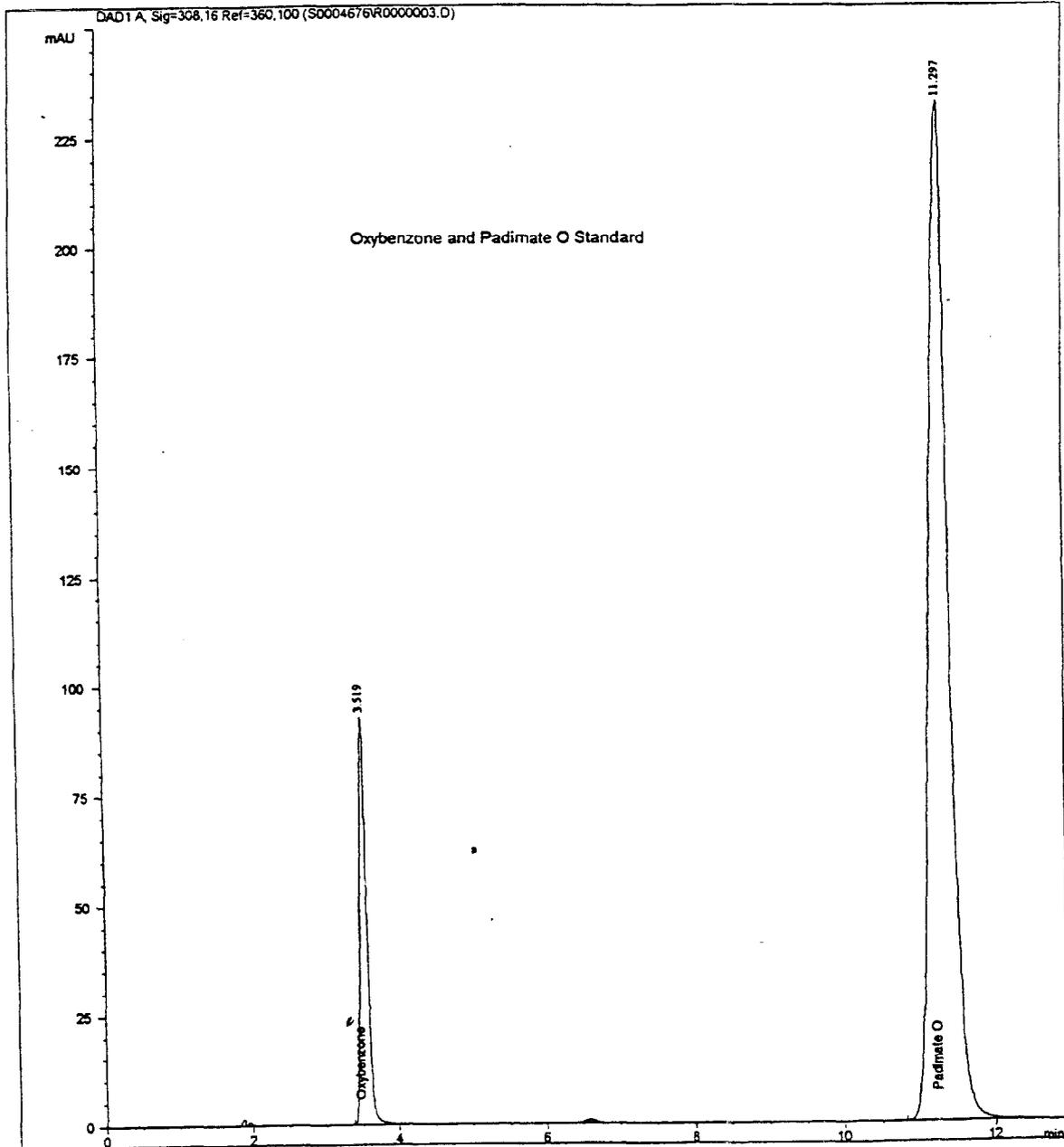


Figure 13. Typical Standard Chromatogram

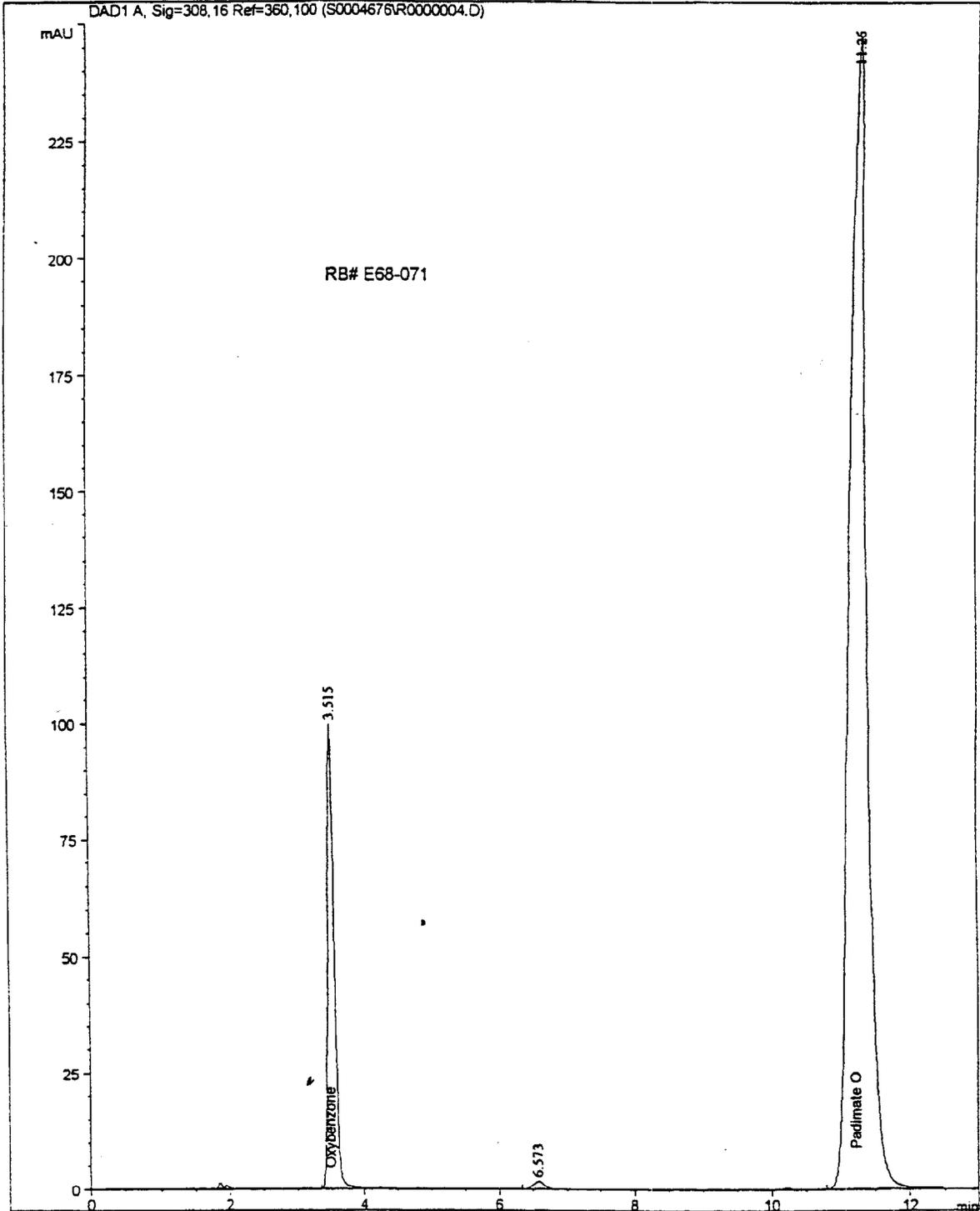


Figure 14. Typical Sample Chromatogram

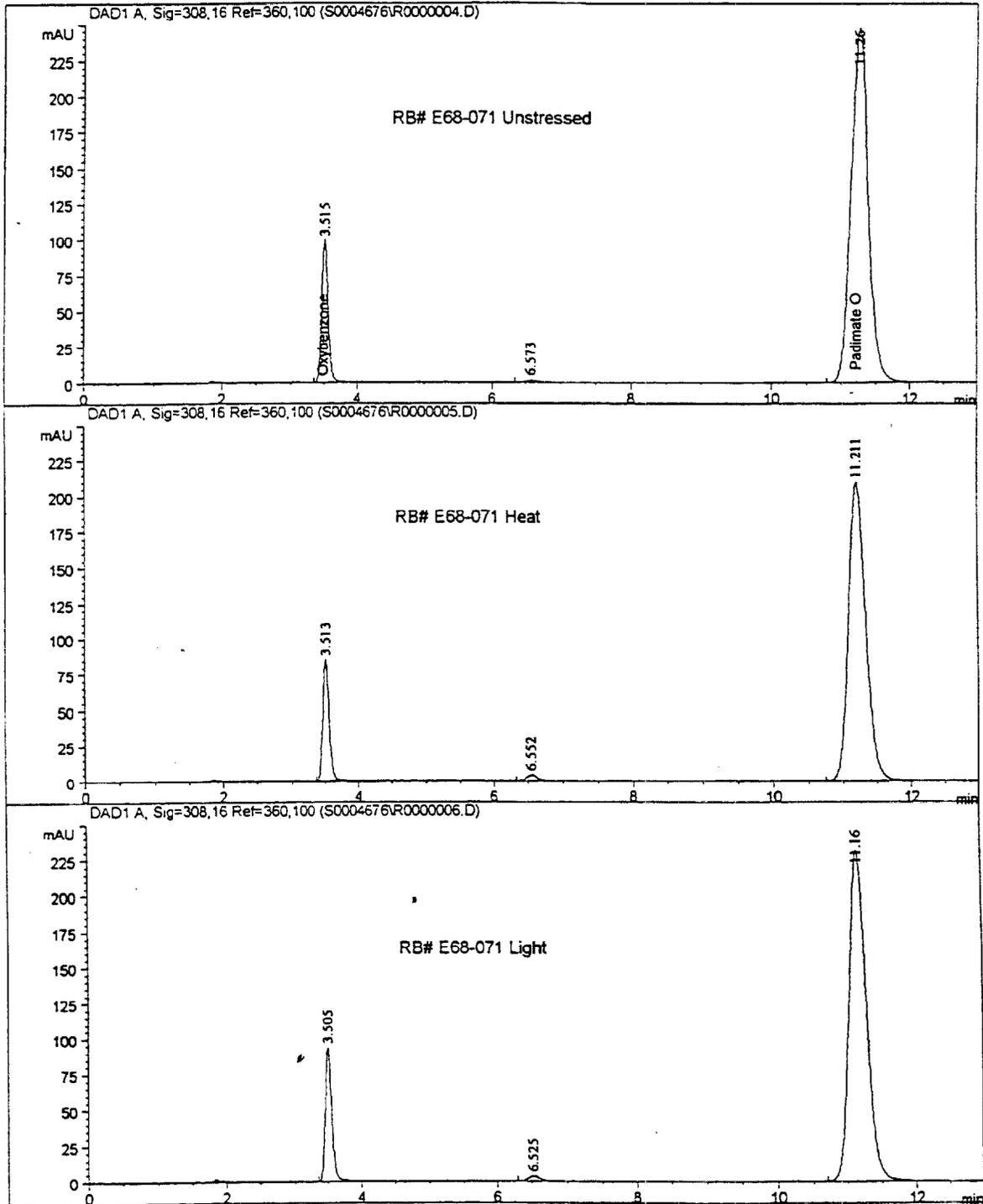


Figure 15. Product, RB# E68-071 Unstressed, Heat, & Light

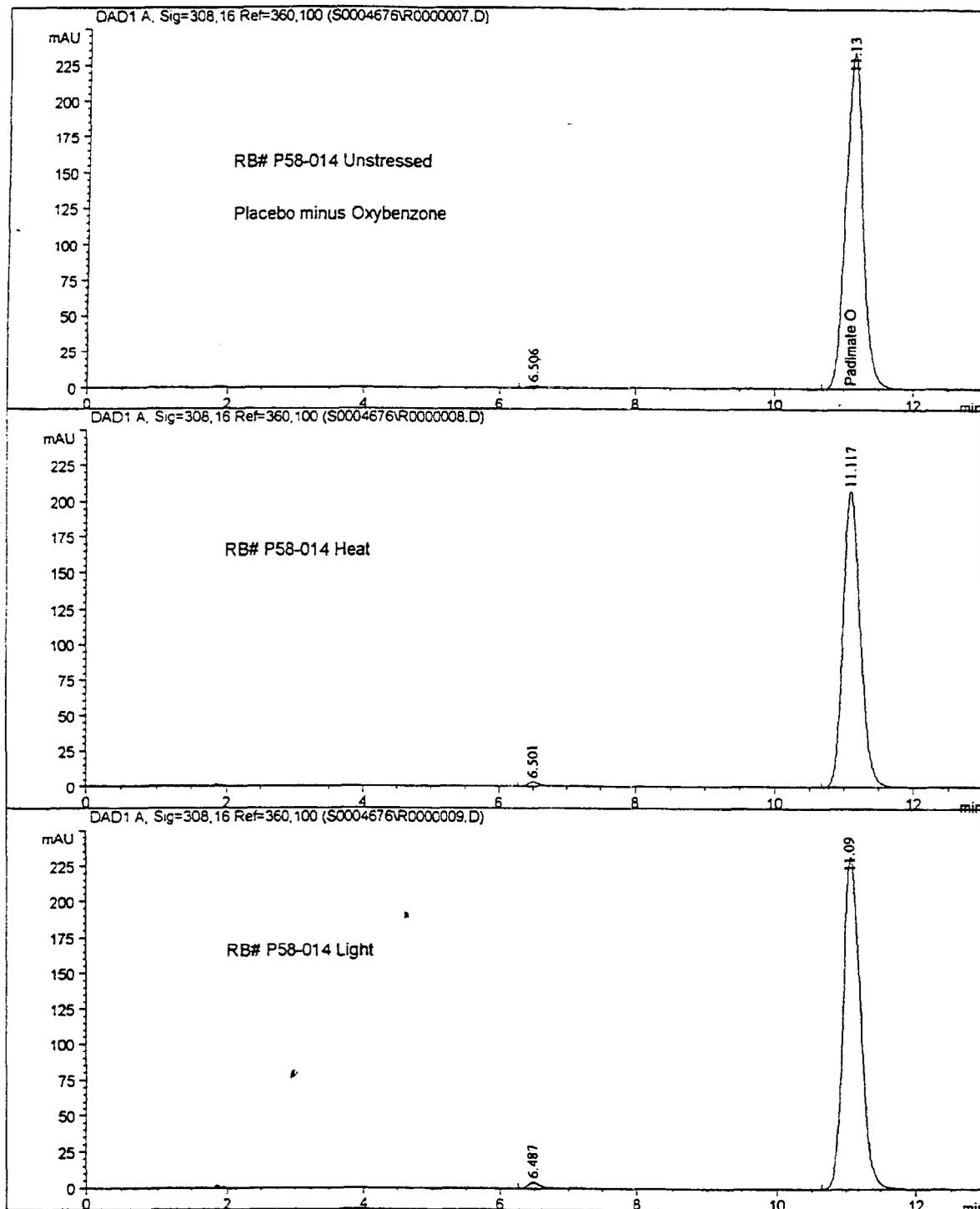


Figure 16. RB# P58-014 Placebo minus Oxybenzone
Unstressed, Heat, & Light

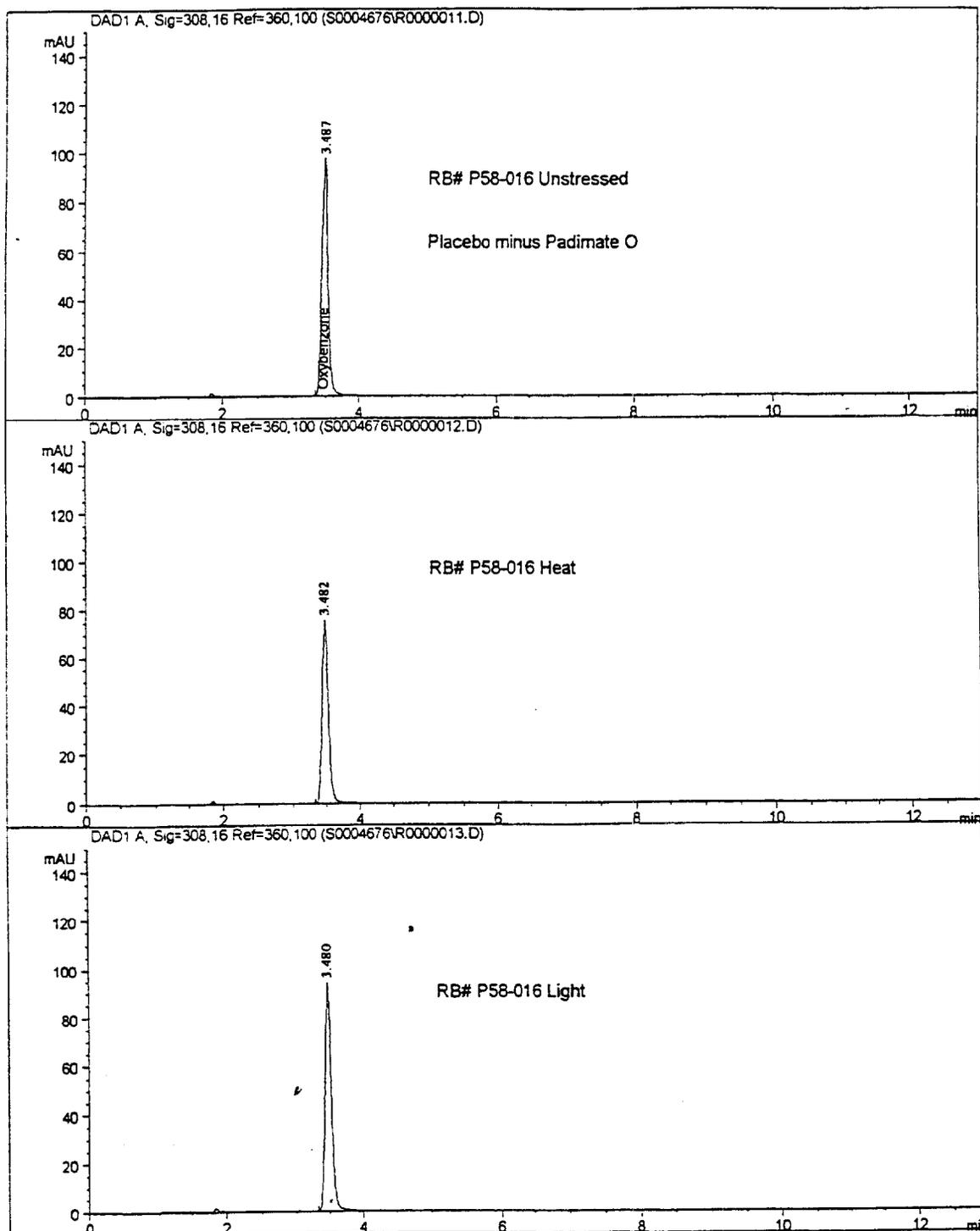


Figure 17. RB# P58-016 Placebo minus Padimate O
Unstressed, Heat, & Light

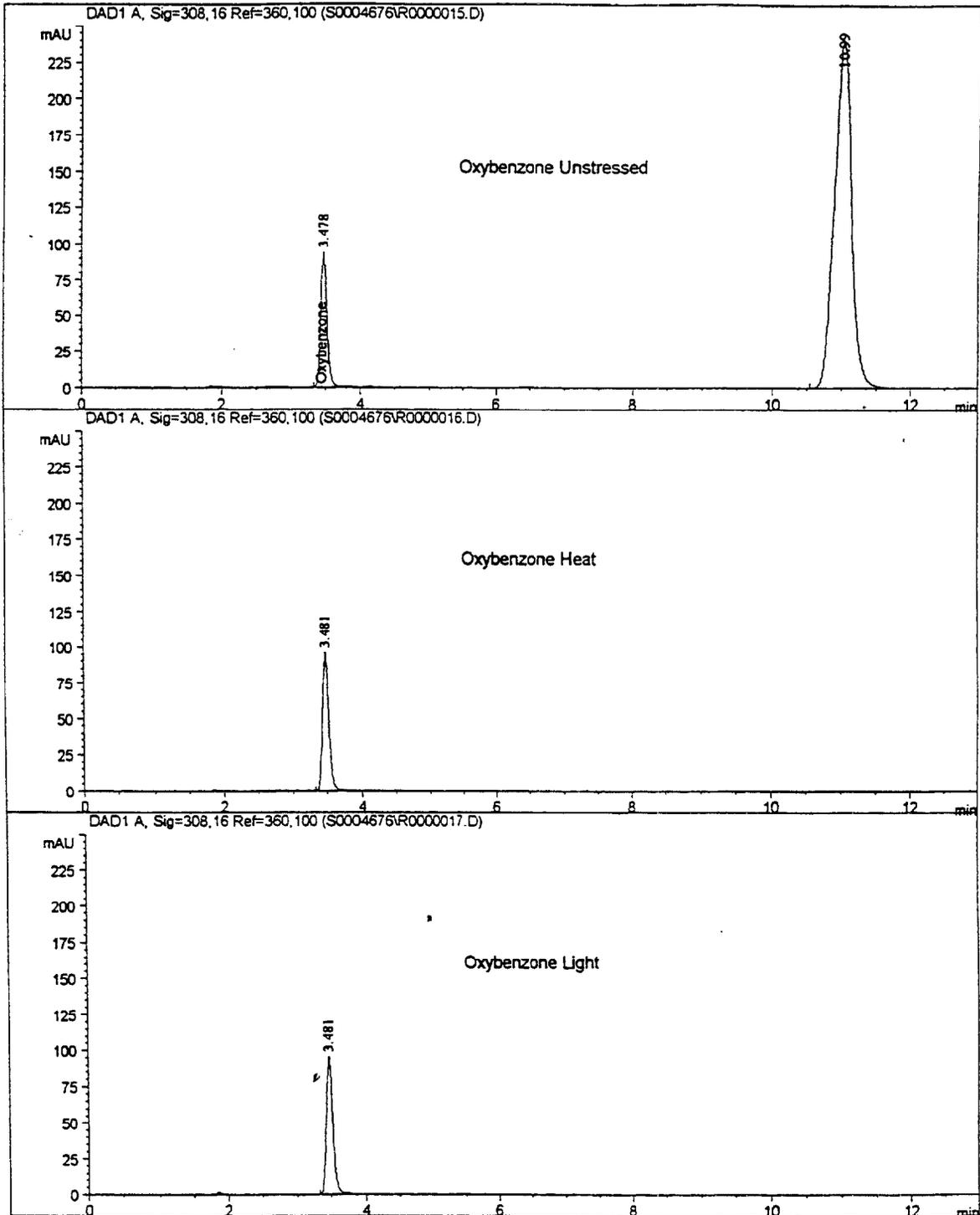


Figure 18. Oxybenzone Unstressed, Heat, and Light Stressed

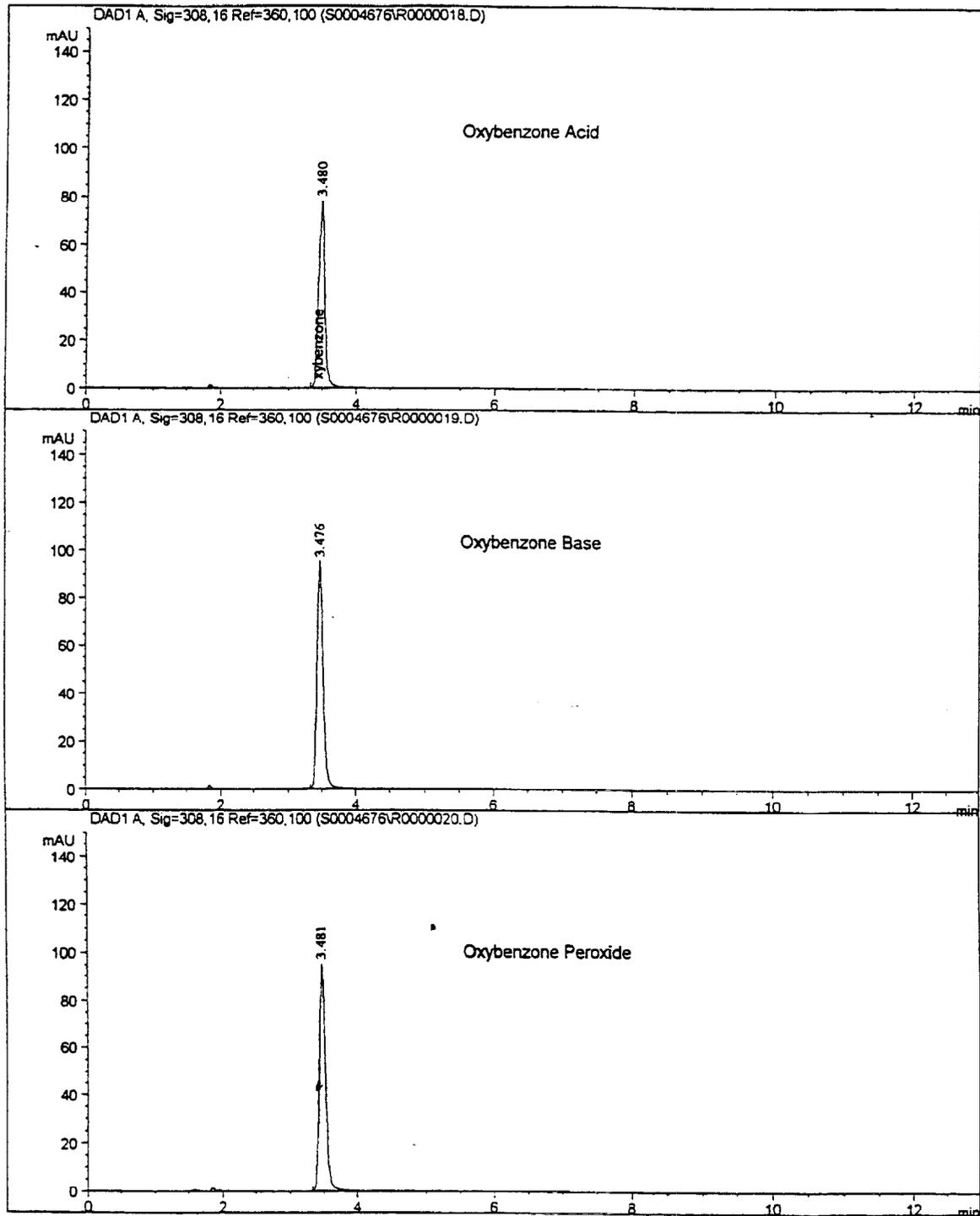


Figure 19. Oxybenzone Acid, Base, and Peroxide Stressed

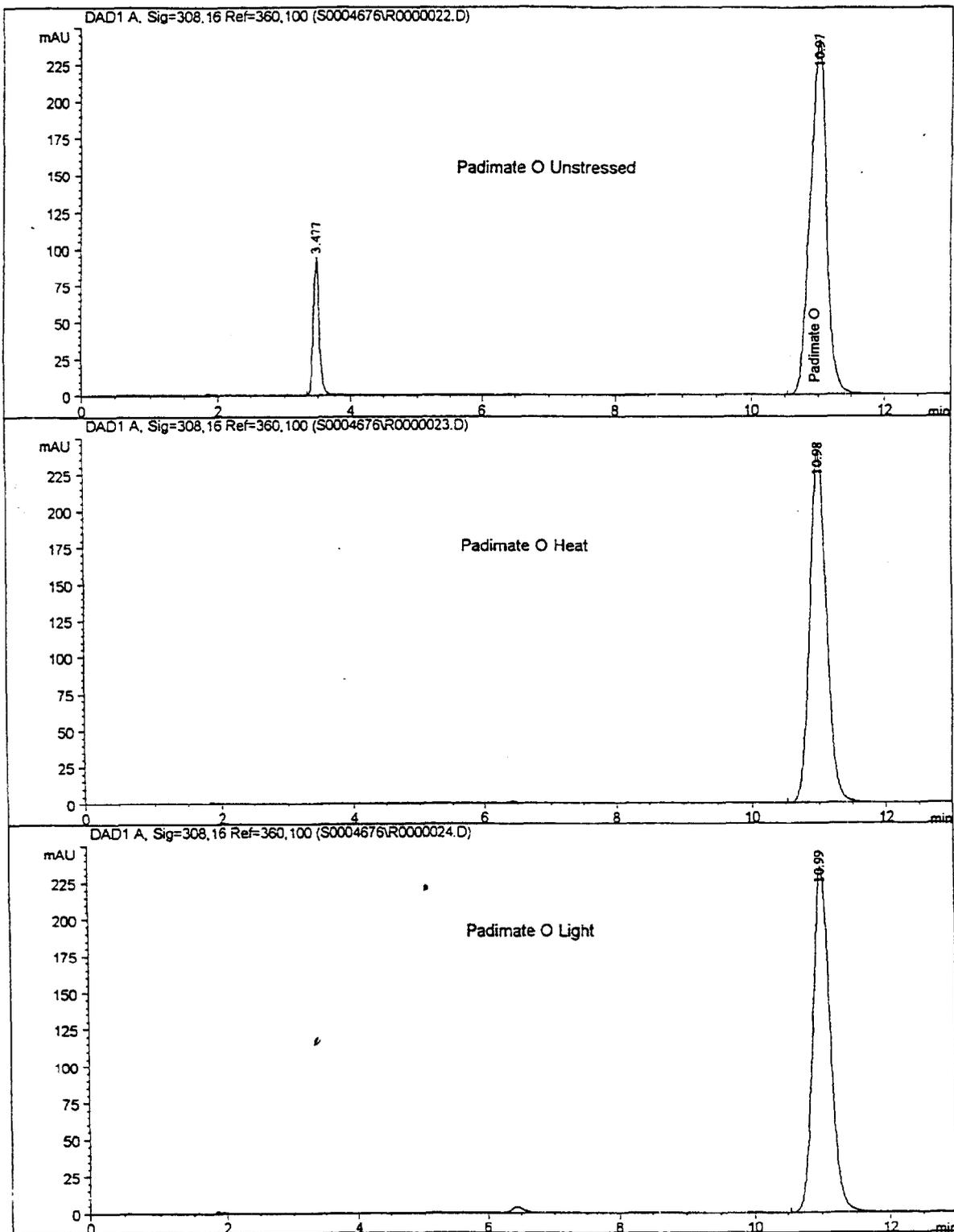


Figure 20. Padimate O Unstressed, Heat, and Light Stressed

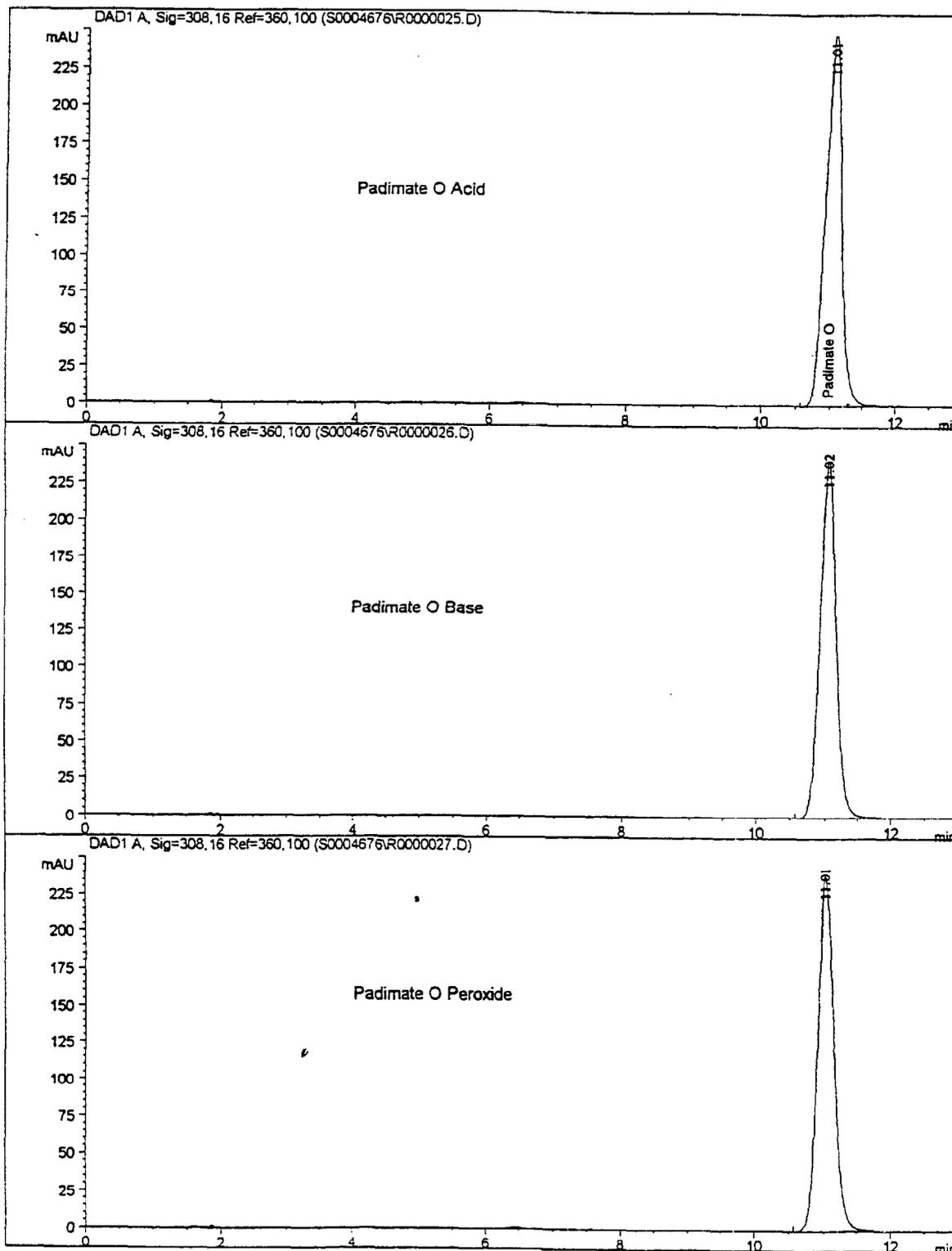


Figure 21. Padimate O Acid, Base, and Peroxide Stressed

Acceptance Criteria:

- The analyte peaks in each sample chromatogram are pure by photodiode array analysis and any excipient, or degradant peaks are resolved from the analyte peaks.

Conclusion:

The purity factors for the analytes are greater than 990, indicating no interferences. No interferences were observed in any of the unstressed or stressed placebo chromatograms. See Table 17 for peak purity factors. All peaks are resolved from the analyte peaks. The analytical method meets the acceptance criteria for specificity.

IV. CONCLUSION

The analytical method for the analysis of Oxybenzone and Padimate O in CTFA SPF-15 Standard Lotion, RB# E68-071 is suitable and valid.

ATTACHMENT 1

Sunscreen SPF 15 – Oxybenzone and Padimate O
Assay (% w/w)

A. Reagents:

1. Acetic Acid, glacial, ACS grade
2. Isopropanol, HPLC grade
3. Methanol, HPLC grade
4. Oxybenzone, Reference Standard
5. Padimate O, Reference Standard

B. Instrumentation:

Equilibrate a suitable liquid chromatograph to the following or equivalent conditions:

Column : Ultrasphere ODS 250 x 4.6 mm (5 μ)
Mobile Phase : 85:15:0.5 Methanol:Water:Acetic Acid
Flow Rate : 1.5 mL/min.
Temperature : Ambient
Detector : UV Spectrophotometer @ 308 nm
Attenuation : As needed
Injection Amount : 10 μ L

C. Mobile Phase Preparation:

Mix 850 mL methanol, 150 mL water and 5.0 mL glacial acetic acid.

D. Standard Preparation:

1. Accurately weigh about 0.50 g of Oxybenzone, Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
2. Accurately weigh about 0.50 g of Padimate O, Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
3. Accurately pipet 3.0 mL of the Oxybenzone stock solution (C.1.) and 7.0 mL of the Padimate O stock solution (C.2.) into a 100-mL volumetric flask. Dilute to volume with isopropanol and mix well. This is your Standard Preparation.

E. Sample Preparation:

1. Accurately weigh approximately 1.0 g of sample into a 50-mL volumetric flask.
2. Add approximately 30 mL of isopropanol and heat with swirling until the sample is evenly dispersed.
3. Cool to room temperature and dilute to volume with isopropanol. Mix well.
4. Pipet 5.0 mL of the sample solution (D.3.) into a 50-mL volumetric flask and dilute to volume with isopropanol. Mix well.

F. System Suitability:

An HPLC equilibrated to the above conditions would be considered suitable. This system would insure that three replicate injections of the Standard Preparation would yield a relative standard deviation of not more than 2.0% calculated on peak areas for Oxybenzone and Padimate O. The system would also ensure a calculated resolution between the Oxybenzone and Padimate O peaks of not less than 3.0.

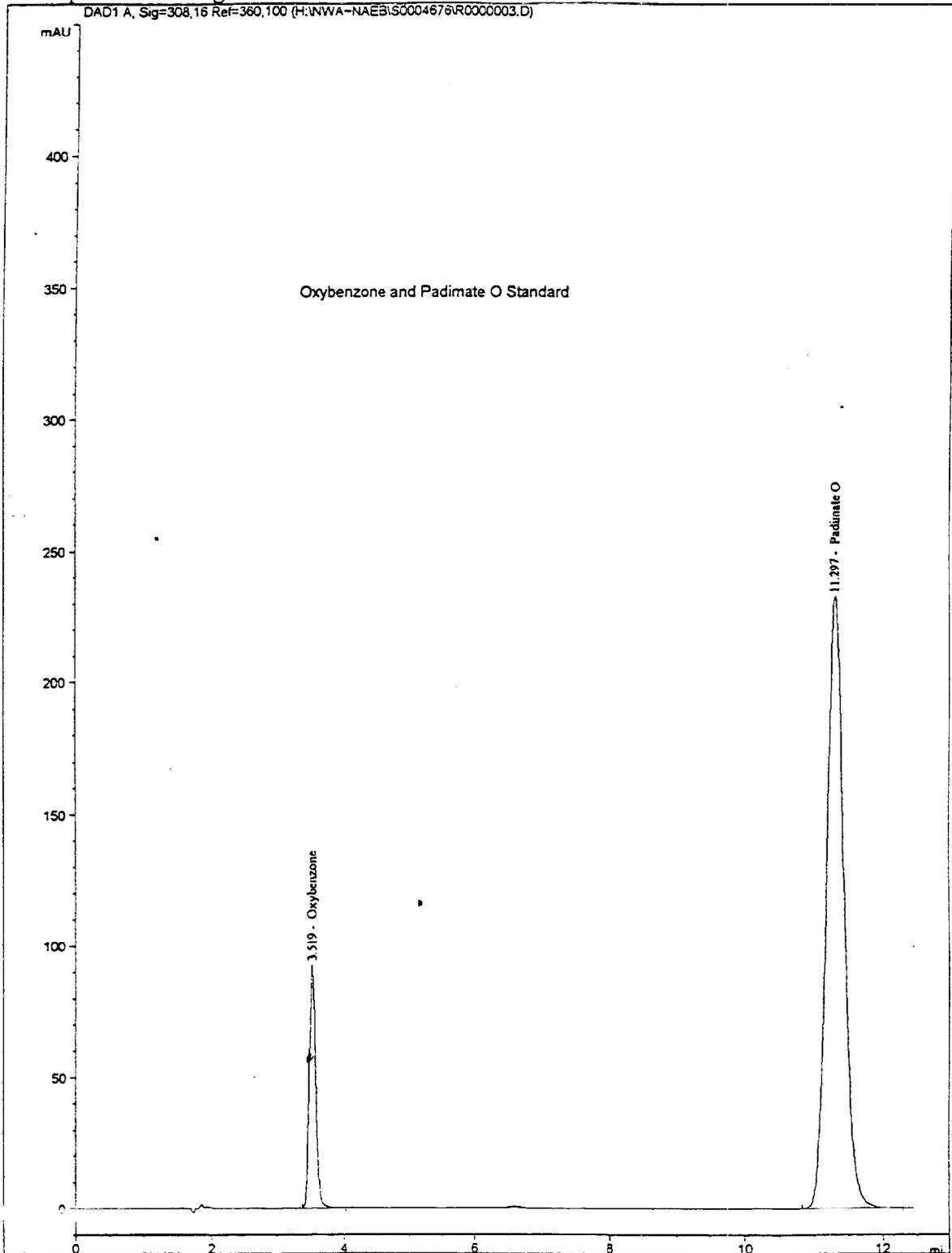
G. Analysis:

1. Inject 10 μ L of the Standard Preparation in triplicate collecting data for about 15 minutes or until the Padimate O peak has completely eluted. Determine if the system meets the suitability criteria as established above. Elution order: (1) Oxybenzone (2) Padimate O.
2. Similarly inject 10 μ L of each Sample Preparation.
3. Calculate the percent of each sunscreen in the sample as follows:

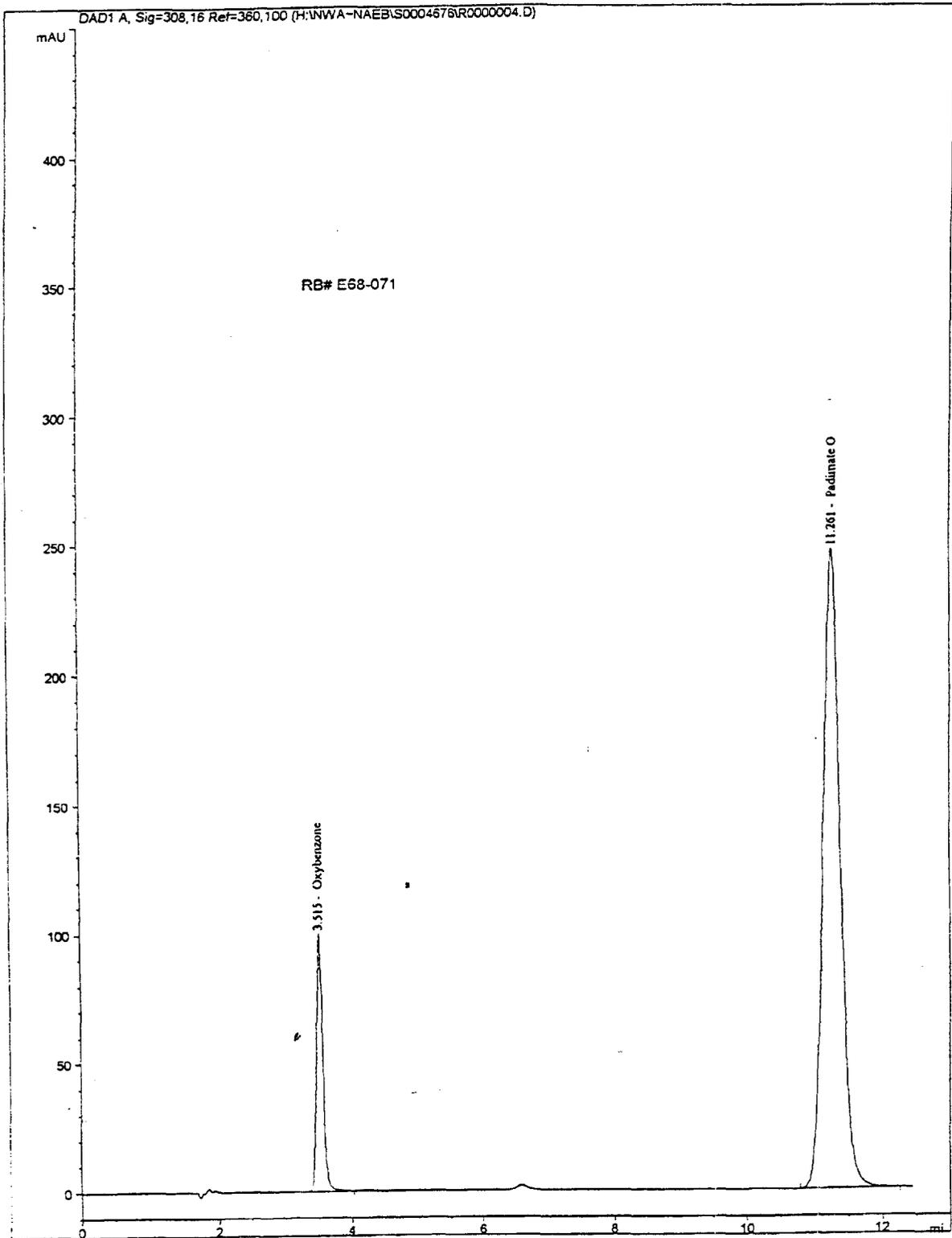
$$\frac{(\text{Smp. Oxybenzone Peak Area})(\text{Std. Oxybenzone Wt. g})(6)}{(\text{Std. Oxybenzone Peak Area})(\text{Smp. Wt. g})} = \text{Oxybenzone \% (w / w)}$$

$$\frac{(\text{Smp. Padimate O Peak Area})(\text{Std. Padimate O Wt. g})(14)}{(\text{Std. Padimate O Peak Area})(\text{Smp. Wt. g})} = \text{Padimate O \% (w / w)}$$

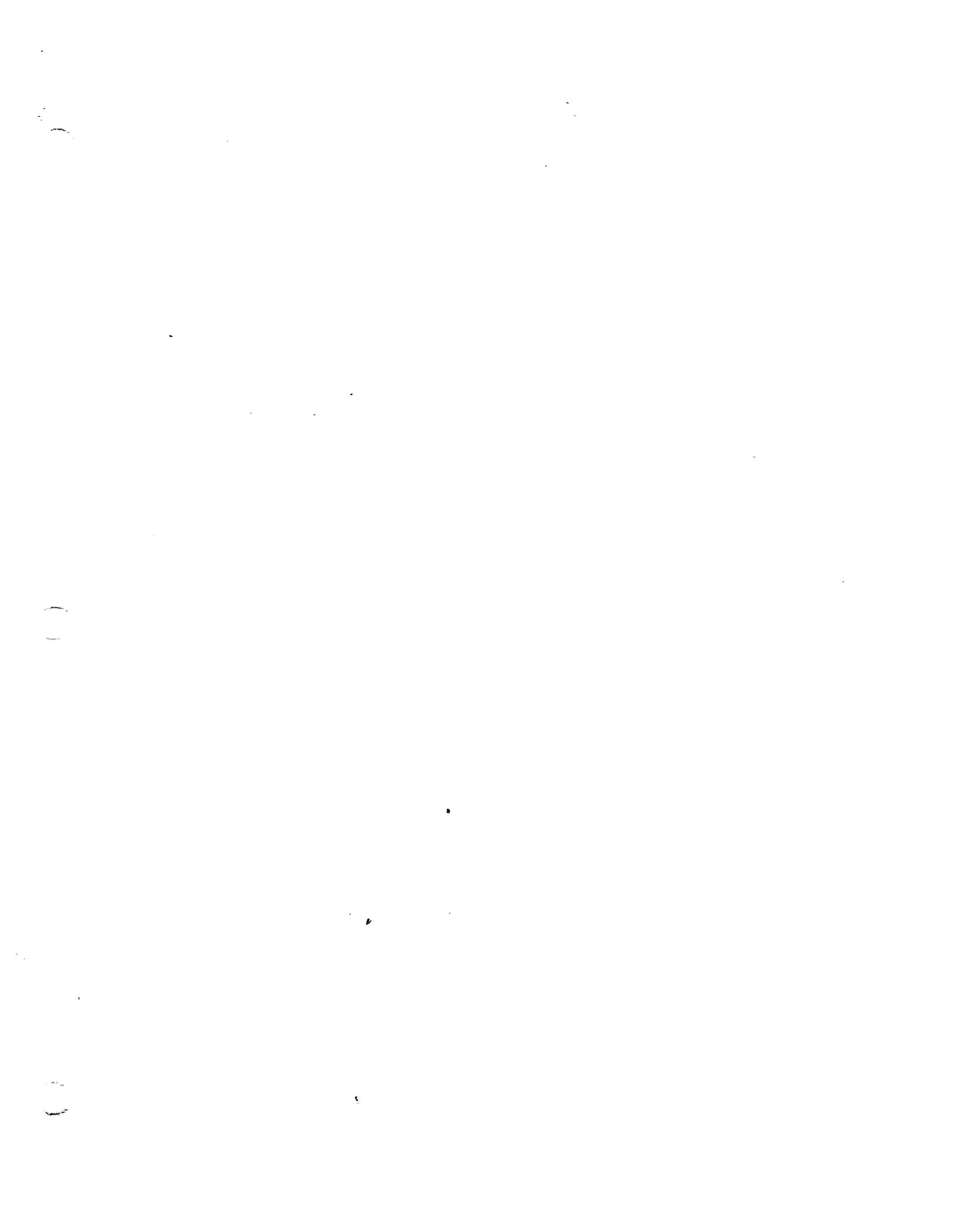
Example Chromatograms:



Typical Standard Chromatogram



Typical Sample Chromatogram



**METHOD VALIDATION SECTION
FOR CTFA SPF 15 STANDARD LOTION**

A. Samples for Method Validation

The following samples are available pursuant to 21 CFR 314.50(e)(1)(i) and will be provided upon request.

Samples for Method Validation may be obtained by contacting:

Dr. C. Rainey
Director, Analytical Research and Development
Schering-Plough HealthCare Products
Memphis, TN 38151
(901)320-2496

Four identical separately packaged subdivisions each containing the samples listed below will be provided:

- One bottle containing 50 grams of CTFA SPF 15 Standard Lotion, Lot Number P58010
- One bottle containing 50 grams of CTFA SPF 15 Standard Lotion PLACEBO without Spectrasorb UV-9 (Oxybenzone), Lot Number P58014
- One bottle containing 50 grams of CTFA SPF 15 Standard Lotion PLACEBO without Escalol 507 (Padimate O), Lot Number P58016
- One bottle containing 15 grams of Spectrasorb UV-9 (Oxybenzone) drug substance, Lot Number ER990078, used in the manufacture of CTFA SPF 15 Standard Lotion, Lot Number P58010
- One bottle containing 15 grams of Escalol 507 (Padimate O) drug substance, Lot Number ER990397, used in the manufacture of CTFA SPF 15 Standard Lotion, Lot Number P58010
- One bottle containing 2 grams of Spectrasorb UV-9 (Oxybenzone) Reference Material, RS018807
- One bottle containing 2 grams of Escalol 507 (Padimate O) Reference Material, RS025805

B. Certificates of Results

The following certificates of analysis are provided.

- a. CTFA SPF 15 Standard Lotion, Lot Number P58010

- b. Spectrasorb UV-9 (Oxybenzone) drug substance, Lot Number ER990078
- c. Escalol 507 (Padimate O) drug substance, Lot Number ER990397
- d. Oxybenzone Reference Material, RS018807
- e. Escalol 507 (Padimate O) Reference Material, RS025805

8% HMS STANDARD LOTION

**Assay Method
SPF 4 Standard Lotion**

Table of Contents

- Attachment 1: Specifications for the 8% Homomenthyl Salicylate Standard Lotion, SPF 4**
- Attachment 2: Formula Composition for the 8% Homomenthyl Salicylate Standard Lotion, SPF 4**
- Attachment 3: Analytical Procedures for the HPLC Method for Assay**
- Attachment 4: Method Validation Report for the Assay**
- Attachment 5: Listing of the Contents for the Method Validation Package for the HPLC Method**

8% HOMOMENTHYL SALICYLATE STANDARD LOTION
Specifications

TEST	SPECIFICATION
Methylparaben	Theoretical: 0.100% w/w Limits: 0.080 to 0.120% w/w
Propylparaben	Theoretical: 0.050% w/w Limits: 0.040 to 0.060% w/w
Homomenthyl Salicylate	Theoretical: 8.00% w/w Limits: 7.44 to 8.56% w/w

8% Homomenthyl Salicylate Standard Lotion

<u>Description</u>	<u>% w/w</u>
Edetate Disodium, USP	0.0500
Homomenthyl Salicylate	8.0000
Lanolin, USP	5.0000
Methylparaben, NF	0.1000
Triethanolamine, NF	1.0000
Propylene Glycol, USP	5.0000
Propylparaben, NF	0.0500
White Petrolatum, USP	2.5000
Stearic Acid, NF	4.0000
Water	74.3000

Sunscreen SPF 4 – Homomenthyl Salicylate
Assay (% w/w)

A. Reagents:

1. Acetic Acid, glacial, ACS grade
2. Isopropanol, HPLC grade
3. Methanol, HPLC grade
4. Homomenthyl Salicylate, Reference Standard

B. Instrumentation:

Equilibrate a suitable liquid chromatograph to the following or equivalent conditions:

Column :	Ultrasphere ODS 150 x 4.6 mm (5 μ) or Ultrasphere ODS 250 x 4.6 mm (5 μ)
Mobile Phase :	85:15:0.5 Methanol:Water:Acetic Acid
Flow Rate :	1.5 mL/min.
Temperature :	Ambient
Detector :	UV Spectrophotometer @ 308 nm
Attenuation :	As needed
Injection Amount :	10 μ L

C. Mobile Phase Preparation

Mix 850 mL methanol, 150 mL of water and 5.0 mL of glacial acetic acid.

D. Standard Preparation:

1. Accurately weigh about 0.50 g of Homomenthyl Salicylate (HMS) Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
2. Accurately pipet 20.0 mL of the HMS stock solution (C.1.) into a 100-mL volumetric flask. Dilute to volume with isopropanol and mix well. This is the Standard Preparation.

E. Sample Preparation:

1. Accurately weigh approximately 2.0 g of sample into a 100-mL volumetric flask.
2. Add approximately 75 mL of isopropanol and heat with swirling until the sample is evenly dispersed.
3. Cool to room temperature and dilute to volume with isopropanol. Mix well.

Sunscreen SPF 4 - Homomenthyl Salicylate
Assay (% w/w)

4. Accurately pipet 25.0 mL of the sample solution (D.3.) into a 100-mL volumetric flask and dilute to volume with isopropanol. Mix well.

F. System Suitability:

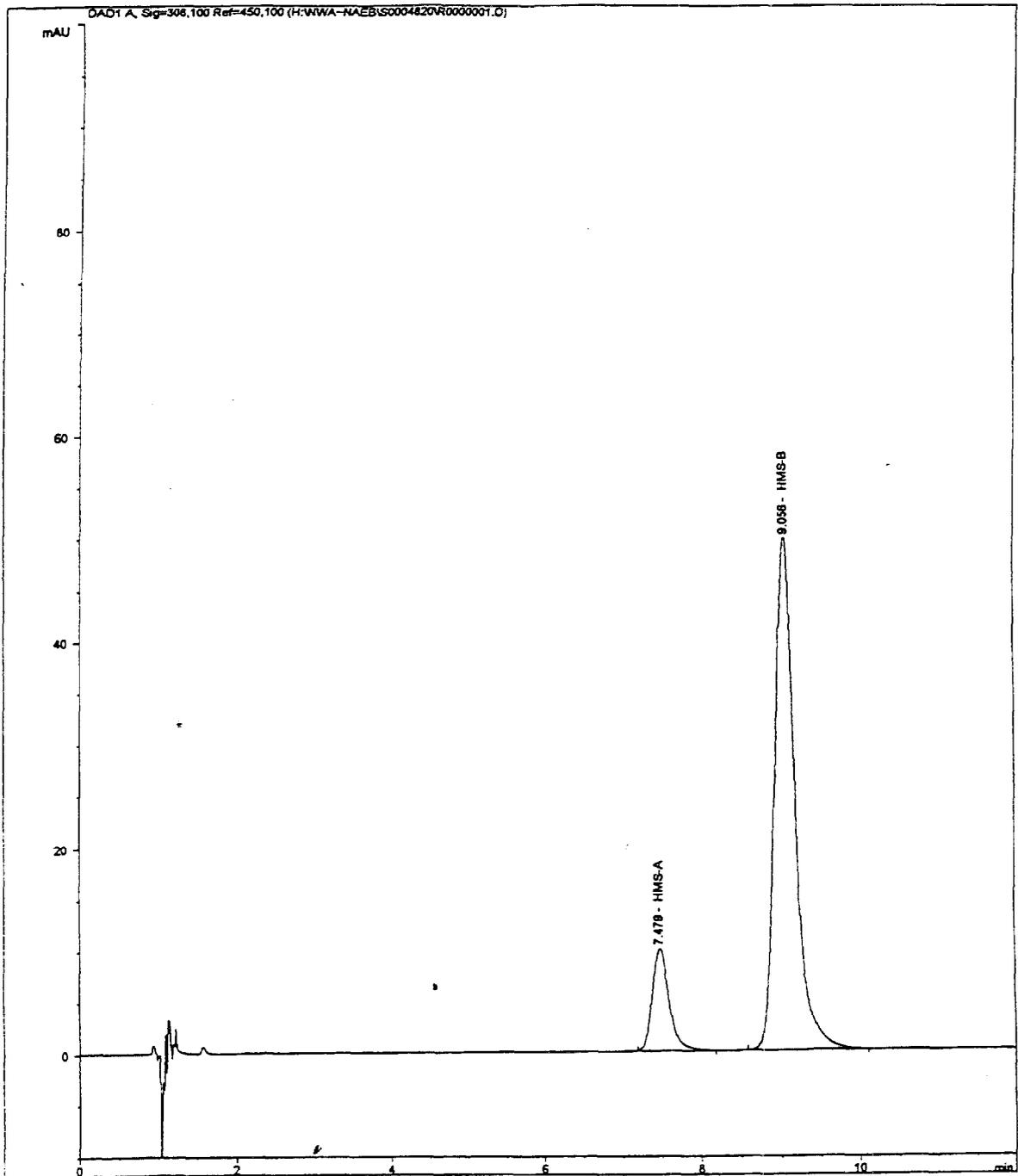
An HPLC equilibrated to the above conditions would be considered suitable. This system would insure that three replicate injections of the Standard Preparation would yield a relative standard deviation of not more than 2.0% calculated on peak areas for HMS. Should a system fail to meet this criterion, adjusting the mobile phase or replacing the column may be necessary to obtain suitable chromatography.

G. Analysis:

1. Inject 10 μ L of the Standard Preparation in triplicate collecting data for about 15 minutes or until both HMS peaks have completely eluted (two isomers). Determine if the system meets the suitability criteria as established above.
2. Similarly inject 10 μ L of each Sample Preparation.
3. Sum the peak areas of the two HMS isomers for each injection and calculate the HMS content in the sample as follows:

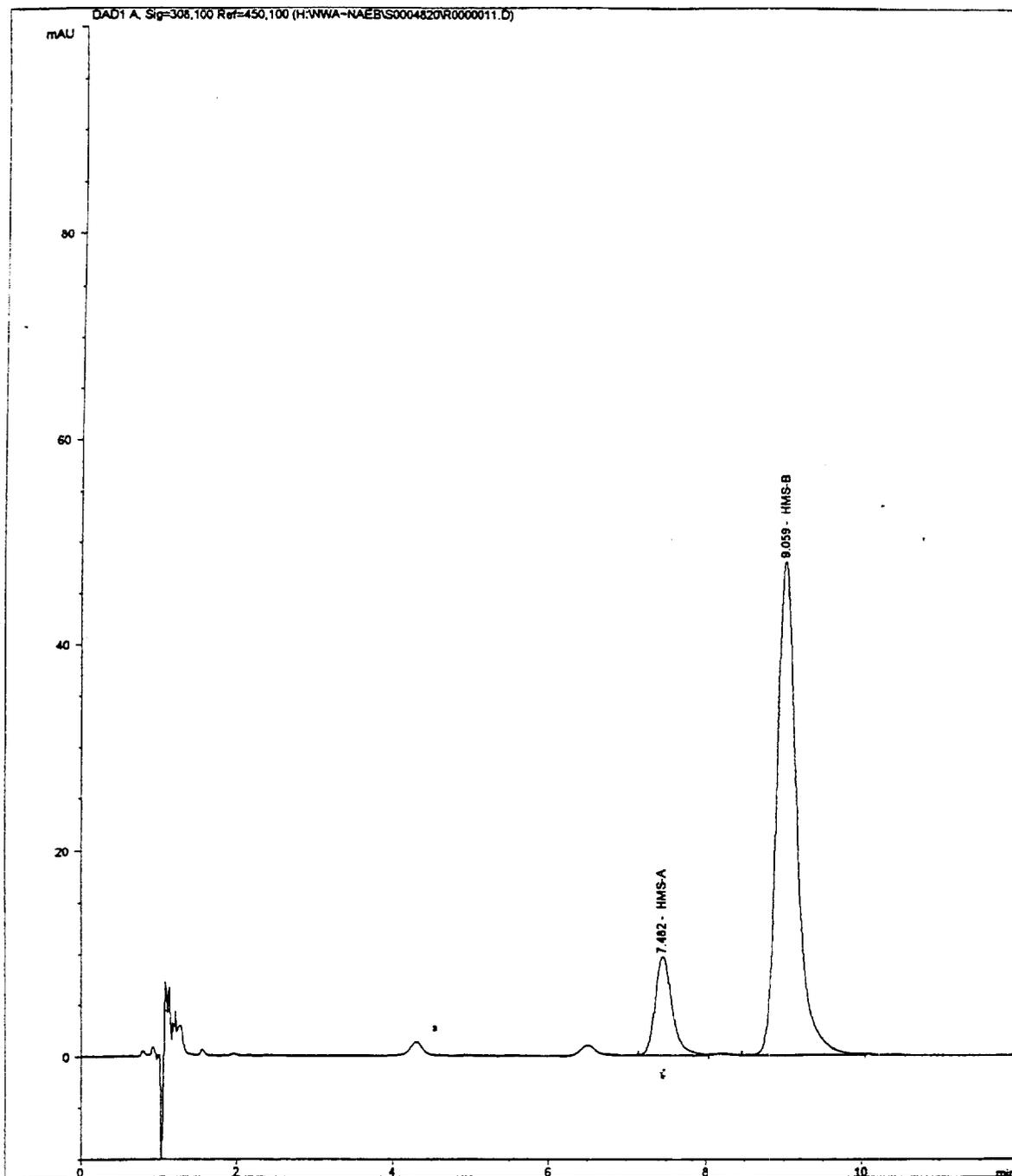
$$\frac{(\text{Total HMS Peak Area for Sample})(\text{Std. Wt. g})(32)}{(\text{Avg. Total HMS Peak Area for Standard})(\text{Smp. Wt. g})} = \text{HMS \% (w/w)}$$

Sunscreen SPF 4 - Homomenthyl Salicylate
Assay (% w/w)



Typical Standard Chromatogram

Sunscreen SPF 4 - Homomenthyl Salicylate
Assay (% w/w)



Typical Sample Chromatogram

SCHERING-PLOUGH HEALTHCARE PRODUCTS
RESEARCH AND DEVELOPMENT
ANALYTICAL RESEARCH

ANALYTICAL METHOD VALIDATION REPORT
Validation Number: 990038

RB# 524-103, 8% Homomenthyl Salicylate Standard Lotion

Assay of Homomenthyl Salicylate

December 17, 1999

007

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I. INTRODUCTION

Purpose:

This document describes the experiments performed to validate the assay procedure used to determine the level of Homomenthyl Salicylate (HMS) in 8% Homomenthyl Standard Lotion.

Formulation:

This formula matrix is a lotion containing the sunscreen HMS at a level of 8%. It also contains lanolin, polyethylene glycol, stearic acid, white petrolatum, triethanolamine, disodium EDTA, USP water and the preservatives, methyl paraben and propyl paraben.

The analytical method for the analysis of HMS is found in ATTACHMENT 1.

The chemical name for Homomenthyl Salicylate (HMS) is 3,3,5-trimethylcyclohexyl salicylate. The empirical formula is $C_{16}H_{22}O_3$ and the structure is in Figure 1.

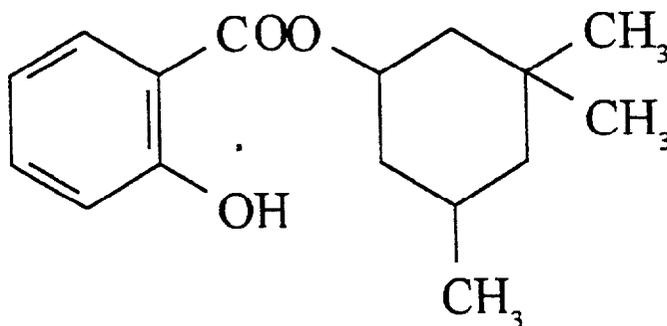


Figure 1. Homomenthyl Salicylate

Method Information:

The proposed analytical method for the assay of Homomenthyl Salicylate in this formula uses high performance liquid chromatography (HPLC) with a C₁₈ reverse-phase column to achieve separation. Detection of the actives is by UV absorbance at a wavelength of 308nm.

A copy of the analytical method for the assay of Homomenthyl Salicylate in this product is presented in ATTACHMENT 1.

This report describes the experiments performed and data generated to validate this analytical method. It demonstrates the suitability of the method to quantitate Homomenthyl Salicylate in this product.

The following experiments were performed for the validation of the analytical method for the assay of Homomenthyl Salicylate in 8% Homomenthyl Salicylate Standard Lotion:

- A. Evaluation of Linearity and Working Concentration Range of the Standard.
- B. Evaluation of System Accuracy and Recovery from Spiked Placebos.
- C. Evaluation of System Precision.
- D. Evaluation of Repeatability.
- E. Evaluation of Reproducibility.
- F. Evaluation of Standard/Sample Stability.
- G. Evaluation of Method Robustness.
- H. Evaluation of Specificity.

II. EXPERIMENTAL

The samples used for this validation included:

- RB# 524-103C, Lot # P58018, 8% Homomenthyl Salicylate Standard Lotion
- RB# 524-012, Lot # P58012, 8% Homomenthyl Salicylate Standard Lotion Placebo without HMS

The reference material used in this validation was an approved standard. All solvents used were HPLC grade.

All equipment used in this validation was in calibration as per appropriate standard operating procedures.

Assay testing was performed in accordance with the analytical method in ATTACHMENT 1.

III. RESULTS

A. Evaluation of Linearity and Working Concentration Range of the Standard

Linearity in an analytical procedure is defined as the ability of an assay to detect a proportional response to increasing or decreasing analyte concentration. The working concentration range for an analytical procedure is that range where test results are directly proportional to the analyte concentration and suitable precision is achieved (less than 2% RSD). For true linear response, the ratio of the detector response to concentration (response factor) will remain constant as concentration changes.

Standard solutions were prepared at 50, 80, 100, 120 and 150% of the theoretical content of homomenthyl salicylate in the working standard. After system suitability was established each level was injected in triplicate.

The response factors (RF) at each level were calculated using the following equation:

$$\text{Response Factor} = \frac{\text{Peak Response}}{\text{Concentration}}$$

The tabulated results are shown in Table 1. The data was analyzed using linear regression analysis with the known concentration (mg/ml) as the independent (X) value and the system response (peak area) as the dependent (Y) value. The linear response (Figure 2) is demonstrated by a high coefficient of determination.

The response factors at each level were averaged. The data was plotted with the standard concentration (expressed as % Theory Added) as the X-value and the mean response factor as the Y-value. Parallel lines were drawn at 2% above and below the response factor at 100% of the theoretical working concentration. This response plot assesses the concentration range where the response factors are consistent within experimental variability. This is defined as the working range. Figure 2 shows that the average response factors in the working concentration range of homomenthyl salicylate are within $\pm 2\%$ of the average response factor at 100% for a range of 50 to 150%.

Table 1. Homomenthyl Salicylate Linearity

% Theory Added	Concentration	Response	Response Factor	AVG RF	%RSD
53.68	0.21471	516.712	2406.56	2404.35	0.08
	0.21471	515.884	2402.70		
	0.21471	516.121	2403.81		
85.88	0.34353	825.600	2403.28	2402.56	0.03
	0.34353	825.090	2401.80		
	0.34353	825.360	2402.58		
107.35	0.42941	1030.700	2400.27	2402.06	0.10
	0.42941	1031.120	2401.25		
	0.42941	1032.580	2404.65		
128.82	0.51529	1243.700	2413.59	2413.88	0.01
	0.51529	1243.910	2414.00		
	0.51529	1243.930	2414.04		
161.03	0.64412	1556.280	2416.13	2413.52	0.09
	0.64412	1553.580	2411.94		
	0.64412	1553.920	2412.47		

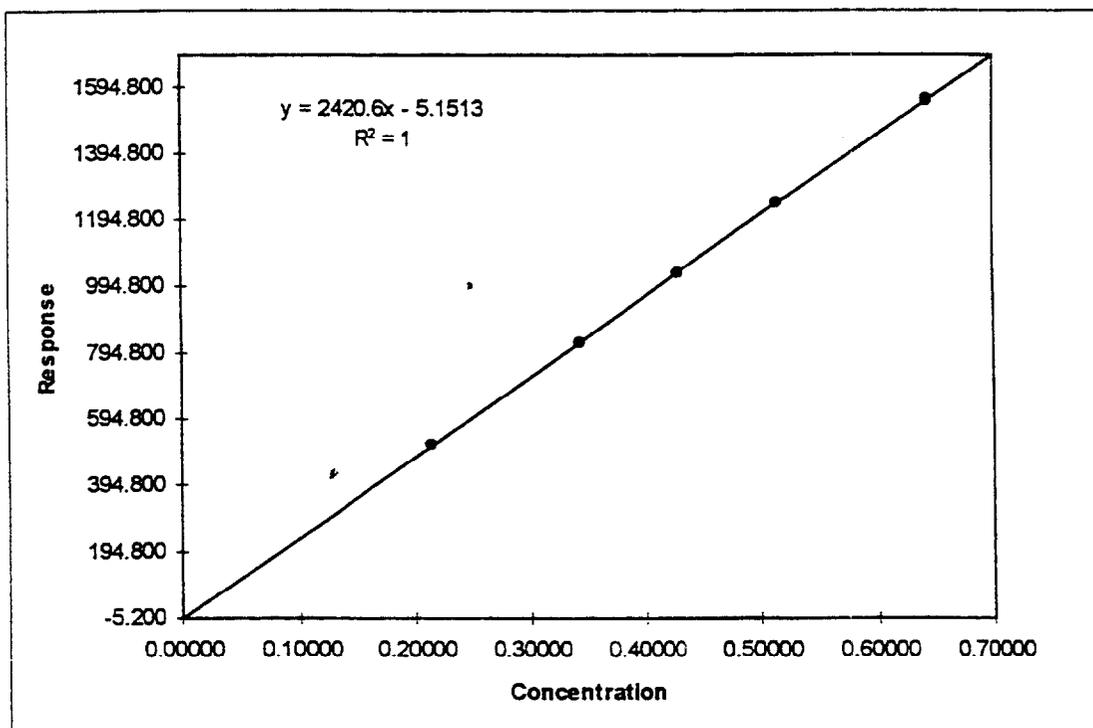


Figure 2. Homomenthyl Salicylate Linearity

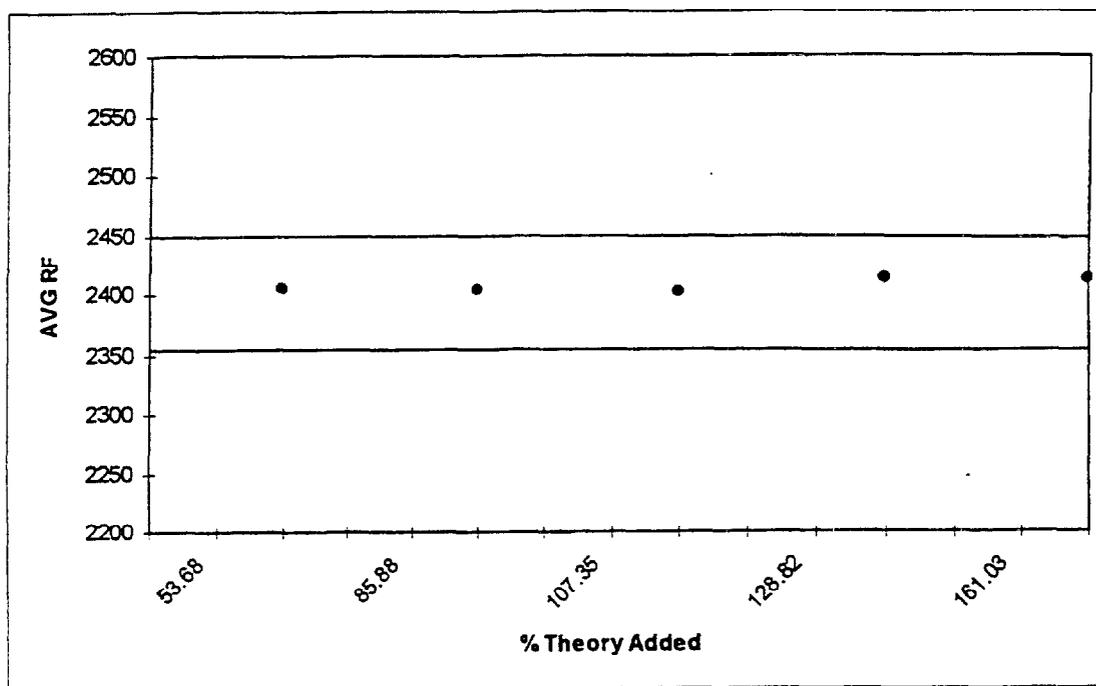


Figure 3. Homomenthyl Salicylate Response Factors

Acceptance Criteria:

- Coefficient of Determination (r^2) = ≥ 0.99
- % RSD for the average Response factor at each level is ≤ 2.0 .
- The average Response Factor at each level is within $\pm 2.0\%$ of the average Response Factor at the 100% level.

Conclusion:

Linear regression analysis was performed on the data using the concentration (mg/ml) as the independent (x) variable and the system response (peak area) as the dependent (y) variable. The calculated regression equation is $Y = 2420.70X - 5.20$, with a coefficient of determination (r^2) of 1.0000. The %RSD of the Response Factors at each level is $\leq 2.0\%$. The Response Factor plot (Figure 3) shows that all values fall within the range of $\pm 2\%$ of the average response factor at 100%. The calculated average response factor at 100% is 2402.06.

The analytical method meets the acceptance criteria for linearity and range.

B. Evaluation of Accuracy and Recovery from Spiked Placebos

Accuracy is defined as the ability of the sample preparation to extract the analyte from the sample matrix to which known amounts of drug substance have been added.

A stock standard was prepared to use as a spiking solution. A dilution from a stock placebo sample preparation was spiked with 0, 50, 100 and 150% of the theoretical amount of homomenthyl salicylate. These samples were analyzed according to the analytical procedure in ATTACHMENT 1. Each sample preparation was injected in triplicate. Table 2 contains the tabulated results showing % Theory Added (spike level), % Theory Found and % Recovery at each level. Figure 4 is a graphical representation of the linear regression.

Table 2. Recovery of Homomenthyl Salicylate from Spiked Placebos
Placebo Lot # P58012

Sample Number	% Theo Added	% Theo Found	% Recovery
0	0.00	0.00	0.00
0	0.00	0.00	0.00
0	0.00	0.00	0.00
50	53.68	53.92	100.45
50	53.68	53.88	100.37
50	53.68	53.86	100.34
100	107.35	107.23	99.89
100	107.35	107.17	99.83
100	107.35	107.24	99.90
150	161.03	161.34	100.19
150	161.03	161.41	100.24
150	161.03	161.49	100.29

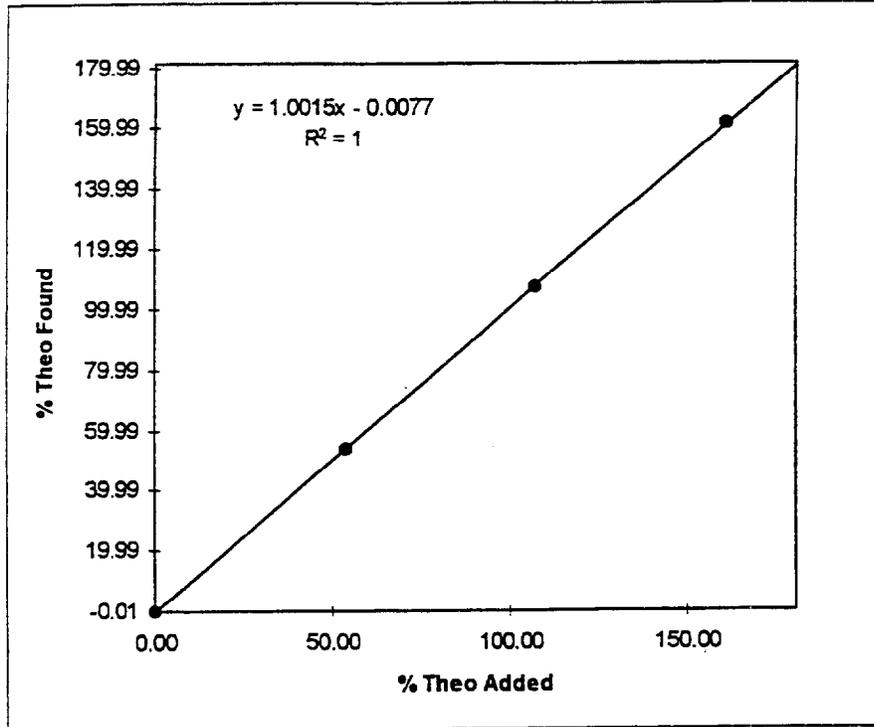


Figure 4. Homomenthyl Salicylate Accuracy

Acceptance Criteria:

- The coefficient of determination (r^2) is ≥ 0.99 .
- At the 95% confidence limits, the slope is 1.0.
- At the 95% confidence limits, the intercept is 0.0.
- The average recovery at each level is 98-102%.
- The percent error due to the intercept at 80 and 120% of theoretical is $-2\% \leq x \leq 2\%$.

Conclusion:

Linear regression was performed on the data using % Theory Added as the independent (x) variable and % Theory Found as the dependent (y) variable.

The linear regression equation is $Y = 1.0015X - 0.0077$ with a coefficient of determination (r^2) of 1.0000. The slope and the intercept are 1.0 and 0.0 respectively, within the 95% confidence limits. The error due to the intercept at 80% of theory is -0.0019 and at 120% of theory is 0.0013%. The average recovery calculated at each level is within the range of 98 to 102% of theory. The method meets the acceptance criteria for accuracy.

C. Evaluation of System Precision:

System precision is established by calculating the %RSD of multiple standard injections performed throughout an analysis. This not only confirms that acceptable precision is obtained initially for the system suitability test, but also that standards repeated throughout an analysis continue to meet system suitability criteria.

During the evaluation of linearity, the standard preparation was injected 6 times (3 standard injections initially and after every 6 sample injections). For the 6 injections over the extended run, the average peak area, standard deviation, and %RSD are shown in Table 3.

Table 3. System Precision

STANDARD	HMS-A	HMS-B	TOTAL HMS
1	1.4277E+02	8.4322E+02	9.8599E+02
2	1.4290E+02	8.4438E+02	9.8728E+02
3	1.4292E+02	8.4416E+02	9.8708E+02
4	1.4296E+02	8.4452E+02	9.8748E+02
5	1.4316E+02	8.4493E+02	9.8809E+02
6	1.4299E+02	8.4457E+02	9.8756E+02
AVERAGE			9.8725E+02
STD DEVIATION			0.7031
% RSD			0.07

Acceptance Criteria:

- The %RSD of the peak areas is ≤ 2.0 .

Conclusion:

The %RSD for the peak areas is 0.07. The method meets the system precision acceptance criterion.

D. Evaluation of Repeatability (Method Precision)

Repeatability was demonstrated by analyzing 6 sample preparations as described in the analytical method in ATTACHMENT 1. The average standard deviation and % RSD were calculated for the assay results. Table 4 contains the data.

Table 4. Homomenthyl Salicylate Repeatability

SAMPLE	RESULT
1	8.147
2	8.102
3	8.109
4	8.143
5	8.077
6	8.031
AVERAGE	8.1015
STD DEVIATION	0.0434
% RSD	0.54

Acceptance Criteria:

- The %RSD of the assay results is $\leq 2.0\%$.

Conclusion:

The % RSD for the assay results is 0.54. The method precision meets the acceptance criteria.

E. Evaluation of Reproducibility

Reproducibility of the method is established to ensure that different laboratories can obtain comparable results within acceptable levels of precision and accuracy. Reproducibility is determined by analyzing the assay data from two different laboratories using different analysts performing replicate sample preparations on different days. The data generated by the laboratories is subjected to statistical treatment in order to calculate the 95% confidence limits for the mean difference between laboratories.

Two replicate samples from a single batch of the product were prepared and assayed in duplicate in LAB 1 (Analytical Validations) by a single analyst on 2 days following the analytical method in ATTACHMENT 1. The product was sent to LAB 2 (Analytical Stability) where a second analyst assayed them as above.

The data was evaluated using the StatGraphics Software and the mean difference between laboratories was determined. Results of this study are summarized in Table 5.

Table 5. HMS Reproducibility

Day 1	Sample	Injection	Lab 1		Lab 2	
			%(w/w)	% Theory	%(w/w)	% Theory
1	1	1	8.150	101.88	8.104	101.30
		2	8.162	102.03	8.142	101.77
	2	1	8.114	101.42	8.123	101.54
		2	8.107	101.33	8.115	101.43
2	1	1	7.974	99.67	8.095	101.18
		2	7.969	99.62	8.069	100.86
	2	1	7.897	98.71	8.095	101.18
		2	7.907	98.84	8.083	101.04
Average			8.04		8.10	
Standard Deviation			0.11		0.02	
%RSD for assay results			1.37		0.25	
%RSD for pooled assay			0.99			
Difference between lab means			0.85			
95% confidence limits			+0.73			

Acceptance Criteria:

- The 95% confidence limits for the mean difference between laboratories is $\leq 4.0\%$.
- The difference between laboratory means is $\leq 3.0\%$.
- The %RSD for the pooled results from both laboratories is ≤ 2.5 .
- The %RSD for the results from each laboratory is ≤ 2.0 .

Conclusion:

Using the Statgraphics Software to analyze data, the 95% confidence limits for the mean difference between laboratories is ± 0.73 . The difference between laboratory means is 0.85. The total variation was distributed as follows: laboratories, 0%; sample preparation, 7.65%; sample injections, 1.55%; and day, 90.80%. The overall mean difference between laboratories is within the acceptable limits. The %RSD for Lab 1 is 1.37 and for Lab 2 is 0.25. The %RSD for the pooled assay results from both laboratories is 0.99. The reproducibility meets the acceptance criteria.

F. Evaluation of Standard/Sample Stability

The stability of the assay standard and sample preparations was determined. A standard and sample were prepared and assayed as described in the method in ATTACHMENT 1. Tables 6 through 7 contain the results of this experiment.

The chromatograms were reviewed for indications of any degradation and stability of the standard and sample solutions.

Table 6. Stability of Standard

	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	4.1589E-07	4.1612E-07	4.1652E-07
% DIFFERENCE	N/A	0.06	0.15

Table 7. Stability of Sample, RB# P58018

	INITIAL	24 HOURS	48 HOURS
RESPONSE FACTORS	5.1048E-06	5.1124E-06	5.1066E-06
% DIFFERENCE	N/A	0.15	0.04

Acceptance Criteria:

- % Difference between response factors obtained on the standard and sample initially and at 48 hours is $\leq 2.0\%$. If the % difference is $> 2.0\%$ the method should specify to prepare fresh daily.

Conclusion:

There was no indication of any analyte degradation in the standard or sample preparation over the 48-hour period. The standard and sample stability meets the acceptance criteria.

G. Evaluation of Method Robustness

Robustness is determined by making small but deliberate changes to method parameters and evaluating their effect on the overall analytical system. Typical liquid chromatographic condition changes include organic strength, pH and flow rate. The changes were investigated using a sample solution.

Any parameter that is found to produce an undesirable effect should be identified in the method as a critical parameter and appropriate cautions included in the method.

The robustness of this method was evaluated by varying the flow rate, the organic strength of the mobile phase, and the acidic strength of the mobile phase. The column was replaced with a second column of the same length (15cm) and also a 25cm column to demonstrate system suitability. See Figures 5 through 8.

The variation in the system and the effect of the changes on the retention times of the homomenthyl salicylate isomers in the sample solution is indicated in Table 8.

Table 8. Method Robustness

Method Parameters	Retention Times	
	HMS-A	HMS-B
Conditions as specified in method	7.40	8.93
Flow Rate = 1.2 ml/min.	8.85	10.64
Flow Rate = 1.8 ml/min.	6.09	7.33
80% Organic in mobile phase	13.58	17.04
90% Organic in mobile phase	4.33	5.00
0.45% Acetic Acid in mobile phase	7.33	8.83
0.55% Acetic Acid in mobile phase	7.32	8.82
CO/21361 - 2 nd 15 cm column	7.10	8.55
CO/25127 - 25 cm column	12.20	14.71

A system equilibrated to the conditions as specified in the method using a second 15cm column yielded a % RSD of 0.44% and using a 25cm column yielded a % RSD of 0.10.

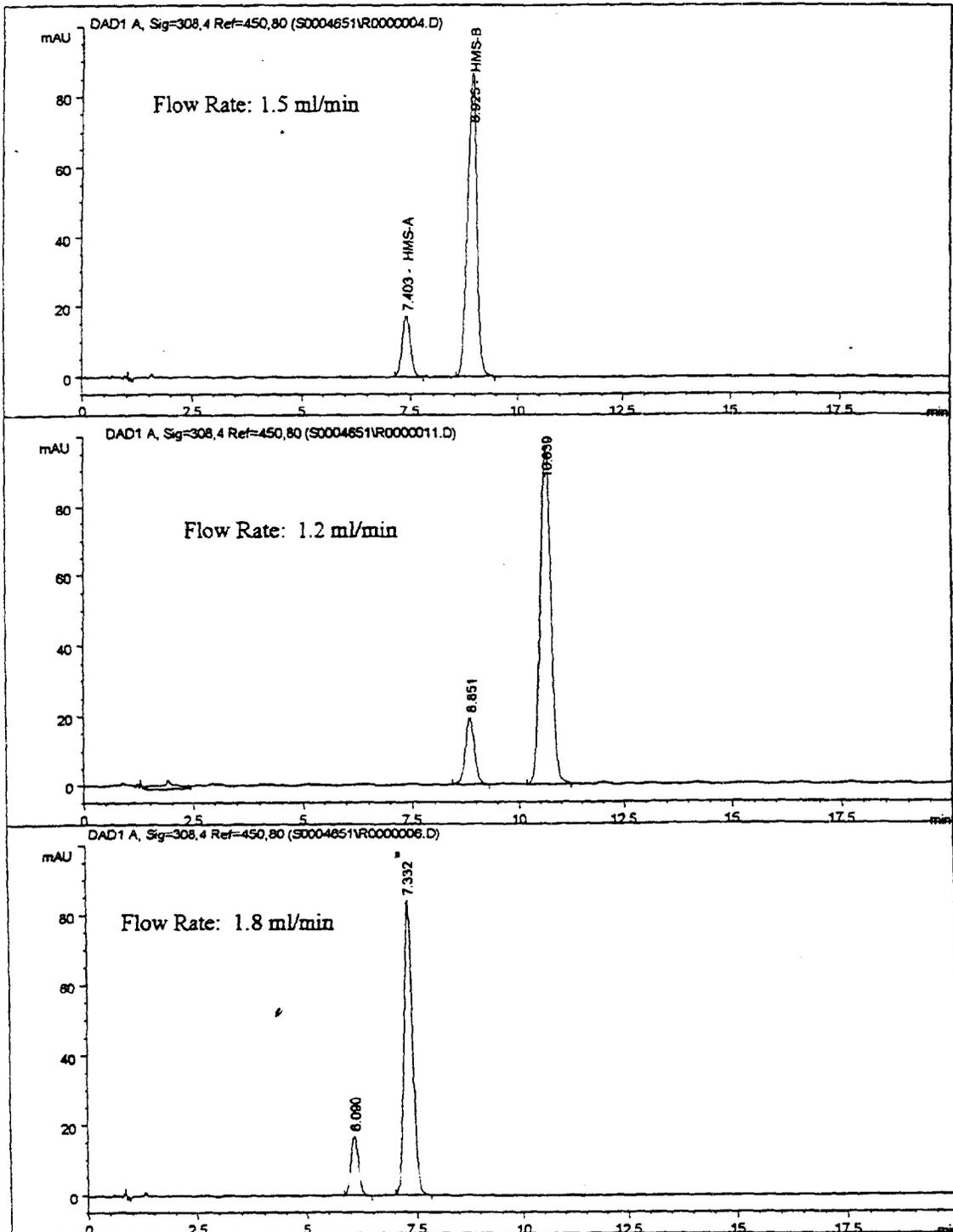


Figure 5. Effect of Flow Rate Variation on Homomenthyl Salicylate

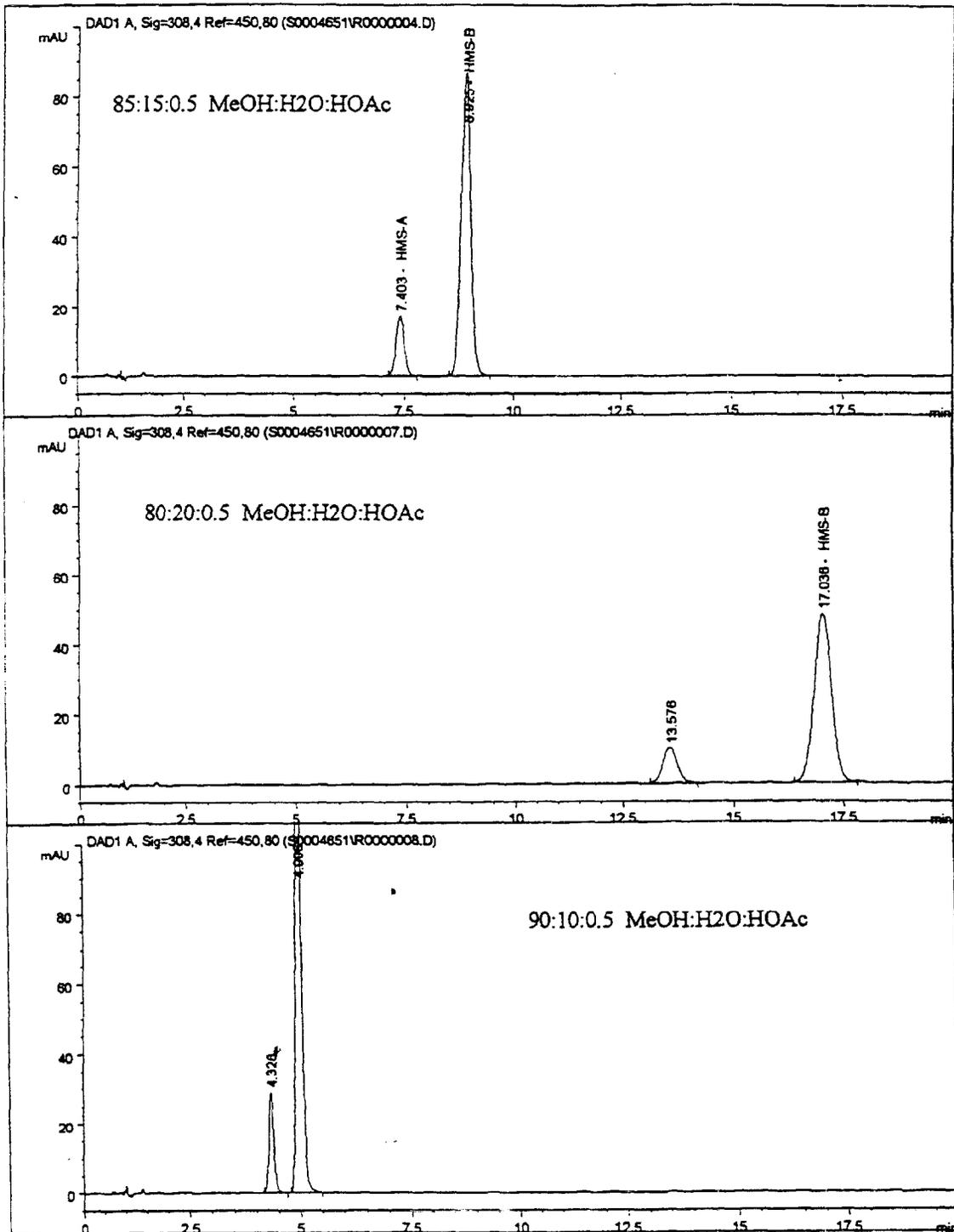


Figure 6. Effect of Organic Variation on Homomenthyl Salicylate

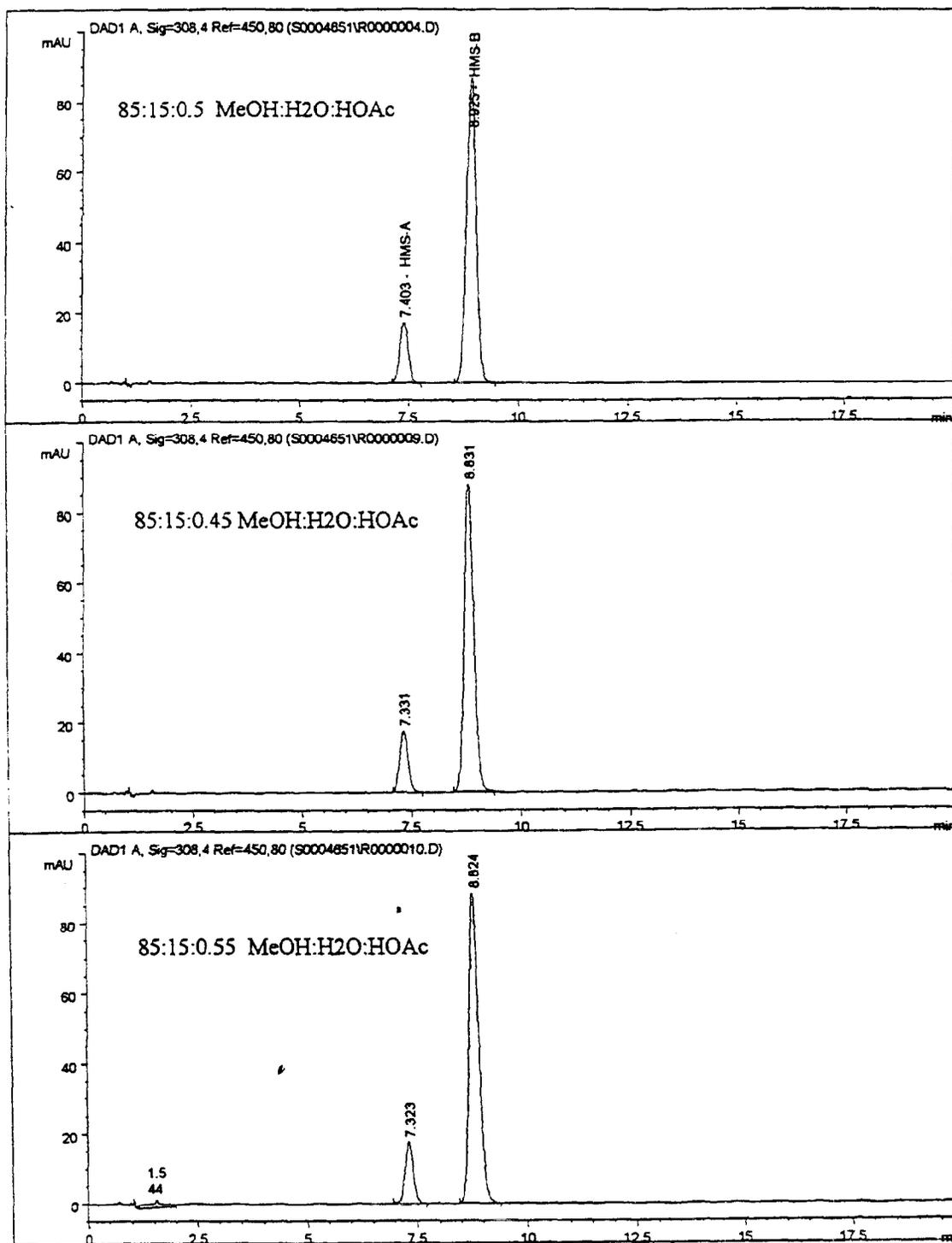


Figure 7. Effect of Acetic Acid Variation on Homomenthyl Salicylate

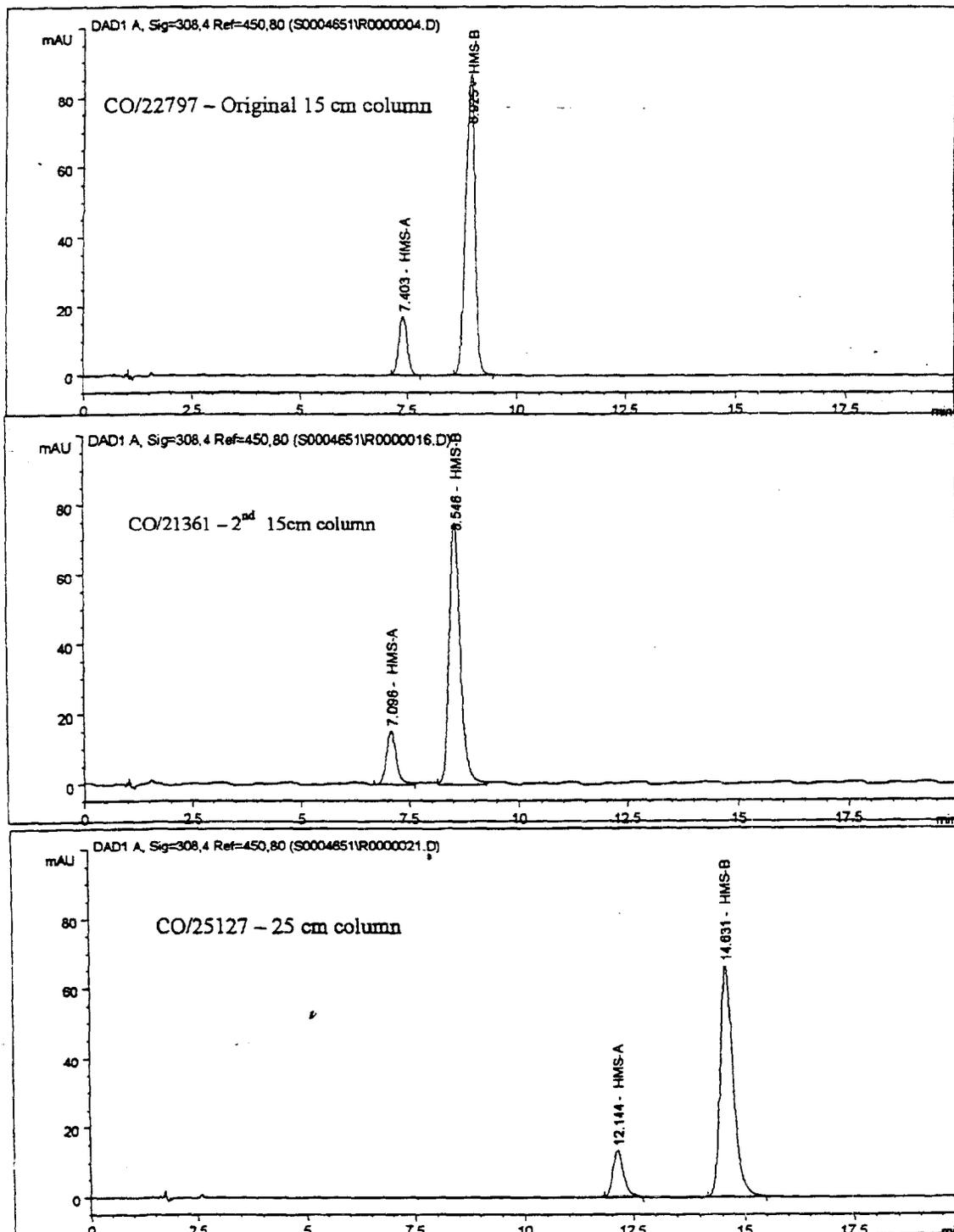


Figure 8. Effect of Column Variation on Homomenthyl Salicylate

Acceptance Criteria:

- Ensure that critical method parameters are identified in the analytical method.

Conclusion:

As can be seen from the data, the organic/aqueous ratio has the most significant effect on the chromatography. The acetic acid content, flow rate, or column length do not cause significant effects in the chromatography. The data indicates suitable ruggedness of the method to small changes.

H. Evaluation of Method Specificity

Specificity is defined as the ability of an analytical method to discriminate the analyte being quantitated without interference from other formula ingredients or degradation products.

To demonstrate the specificity of the analytical method, the formula and the placebo (product less active drug substance) were exposed to heat and light conditions. All heat stress experiments were performed at 60°C in a suitably calibrated oven for two weeks. The light stress experiments were carried out in a light cabinet calibrated to ensure a light density of 1400 footcandles (15,000 lux) for two weeks. Acid stressing was carried out by adding 0.1N HCl to the analyte and heating the sample on a steam bath followed by neutralization. Base stressing was done by adding 0.1N NaOH to the analyte and heating the sample on a steam bath followed by neutralization. The analyte was subjected to oxidative conditions by adding 3% hydrogen peroxide to the analyte and heating in a 40°C water bath.

All stressed and unstressed samples were assayed using the Hewlett-Packard diode array detector according to the method as described in ATTACHMENT 1.

Peak purity of homomenthyl salicylate was determined and chromatograms and spectra of the stressed and unstressed samples were examined for interference. Purity factors were evaluated. The purity value for each spectrum is calculated using the average spectrum of five selected spectra across the peak. If three sequential spectra are outside the purity threshold, the peak is classed as exceeding the limits, and the impure spectra are used for the Purity factor calculation. A numerical value of 1000 indicates a perfect match of spectra generated from the upslope, apex, and downslope of the analyte peak. If this match is 990 or greater, this indicates the peak is pure. Table 9 contains peak purity data. The percent recovery is also reported.

The % Recovery was not recorded for the heat-stressed product due to the lotion liquefying during the heat stressing. The sample was no longer uniform, but the analyte peaks were pure. The placebo also separated.

The purity factors of all samples were above 999.9, indicating purity. This was further substantiated by visually inspecting the data.

Figure 9 is a typical standard chromatogram. Figure 10 is a typical sample chromatogram. Figures 11 through 14 are chromatograms for stressed and unstressed materials.

Table 9. Purity Factors of Unstressed and Stressed Materials

Sample	Stress Condition	Purity Factors		% Recovery
		HMS-A	HMS-B	
Lot # P58018 (Validation Batch)	Unstressed	999.980	999.991	100.80
	Heat	999.944	999.920	N/A
	Light	999.977	999.989	100.57
Lot # P58012 (Placebo w/o HMS)	Light	N/A	N/A	N/A
	Heat	N/A	N/A	N/A
	Unstressed	N/A	N/A	N/A
Analyte Lot# 7Y0416 RS/23077	Unstressed	999.987	999.988	99.44
	Light	999.990	999.987	99.33
	Heat	999.989	999.986	99.41
	Acid	999.986	999.984	97.72
	Base	999.984	999.985	95.00
	Peroxide	999.988	999.988	99.65

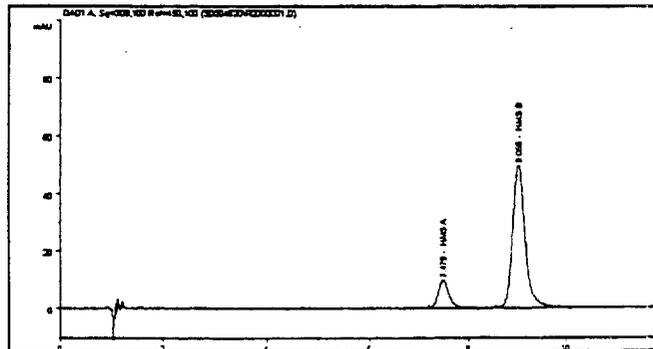


Figure 9. Typical Standard Chromatogram

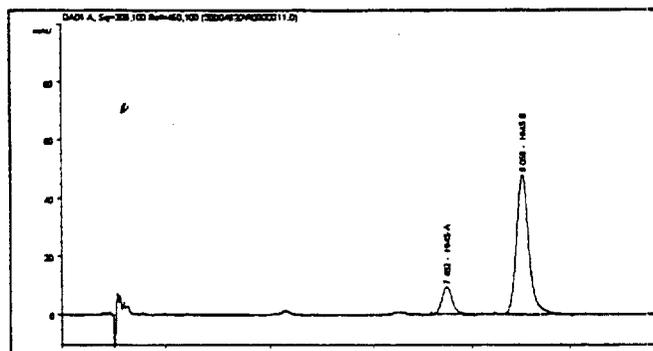


Figure 10. Typical Sample Chromatogram

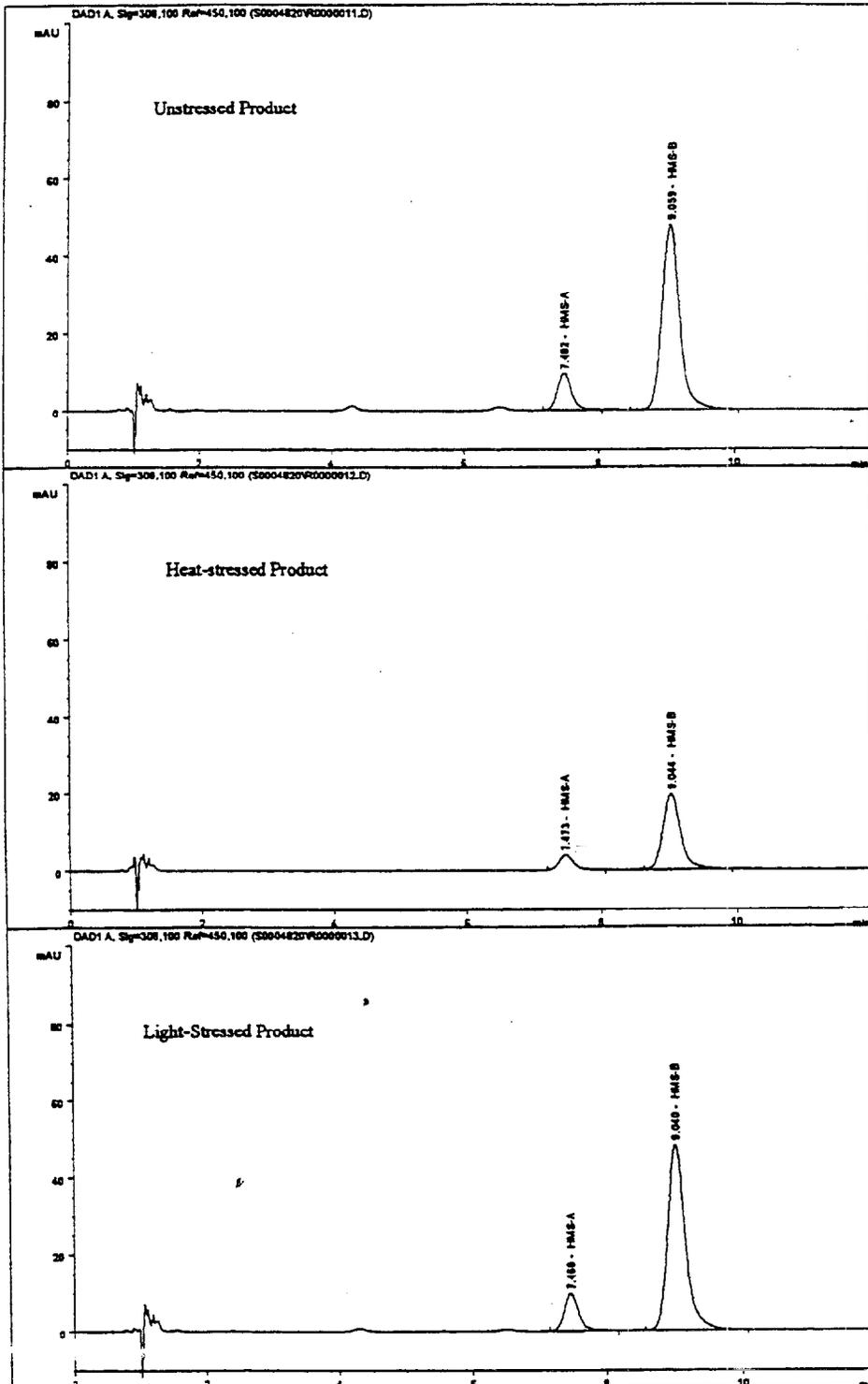


Figure 11. RB# P58018 Unstressed, Heat, & Light Product

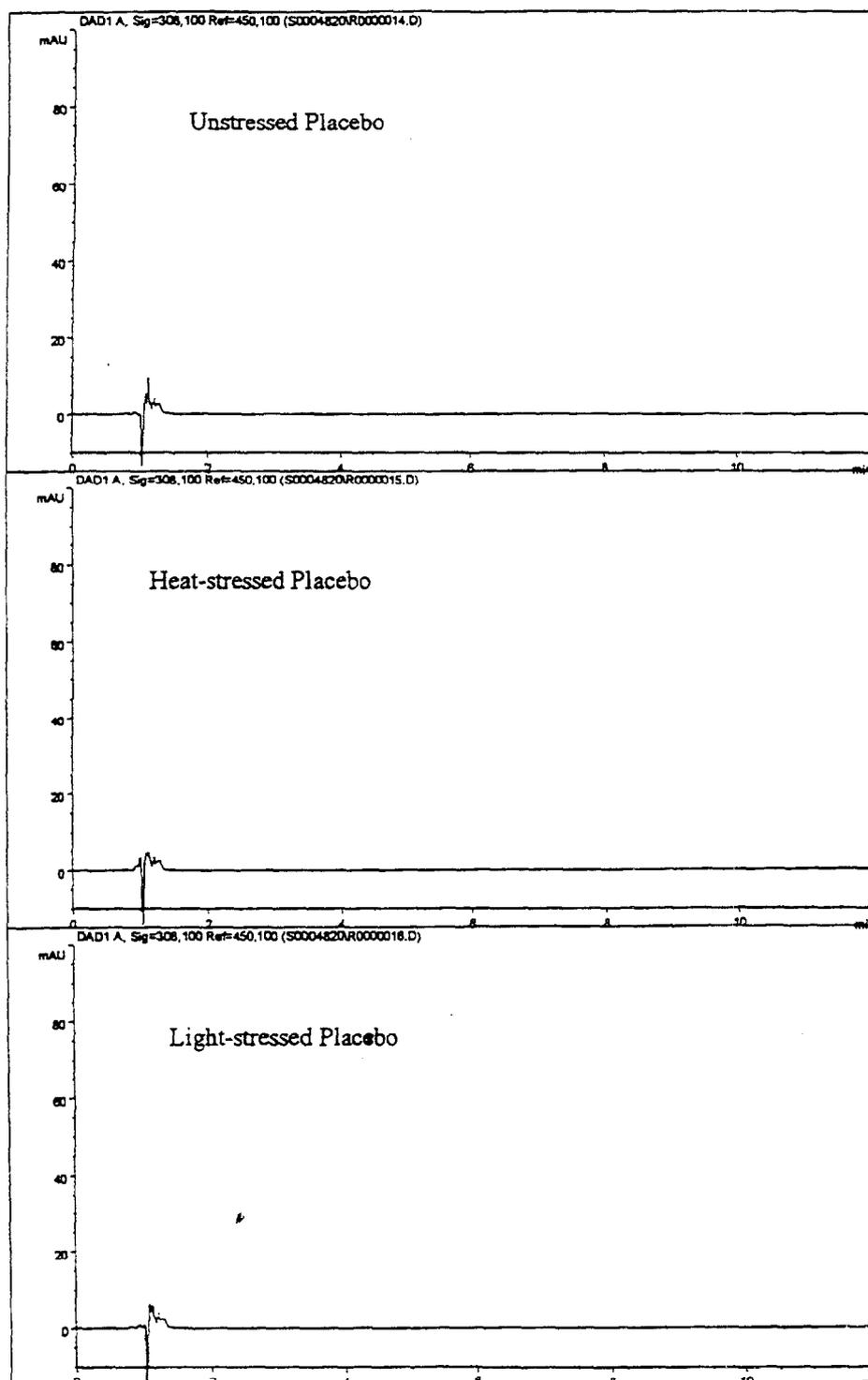


Figure 12. RB# P58012 Unstressed, Heat, & Light Placebo

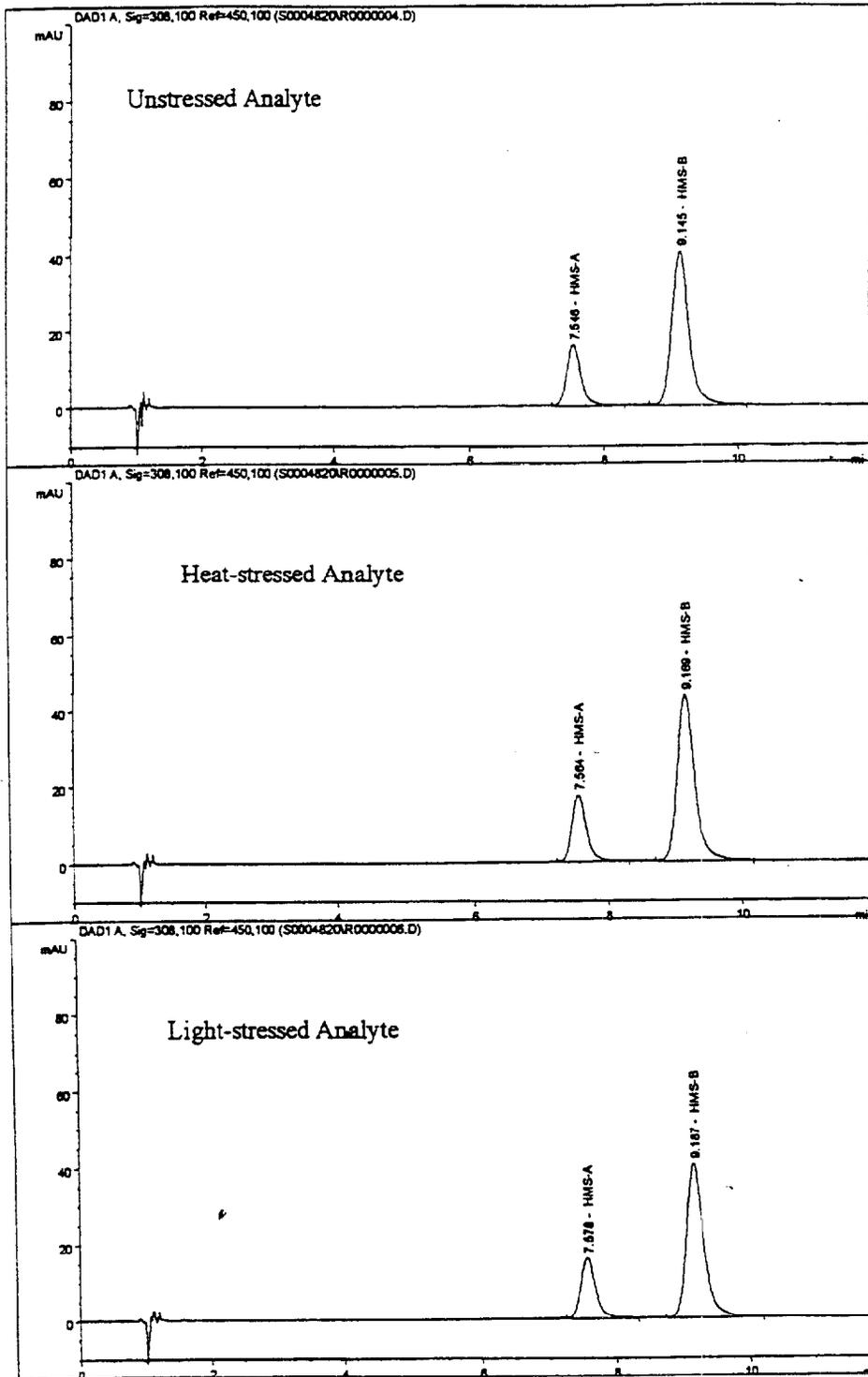


Figure 13. Unstressed and Physically Stressed Analyte

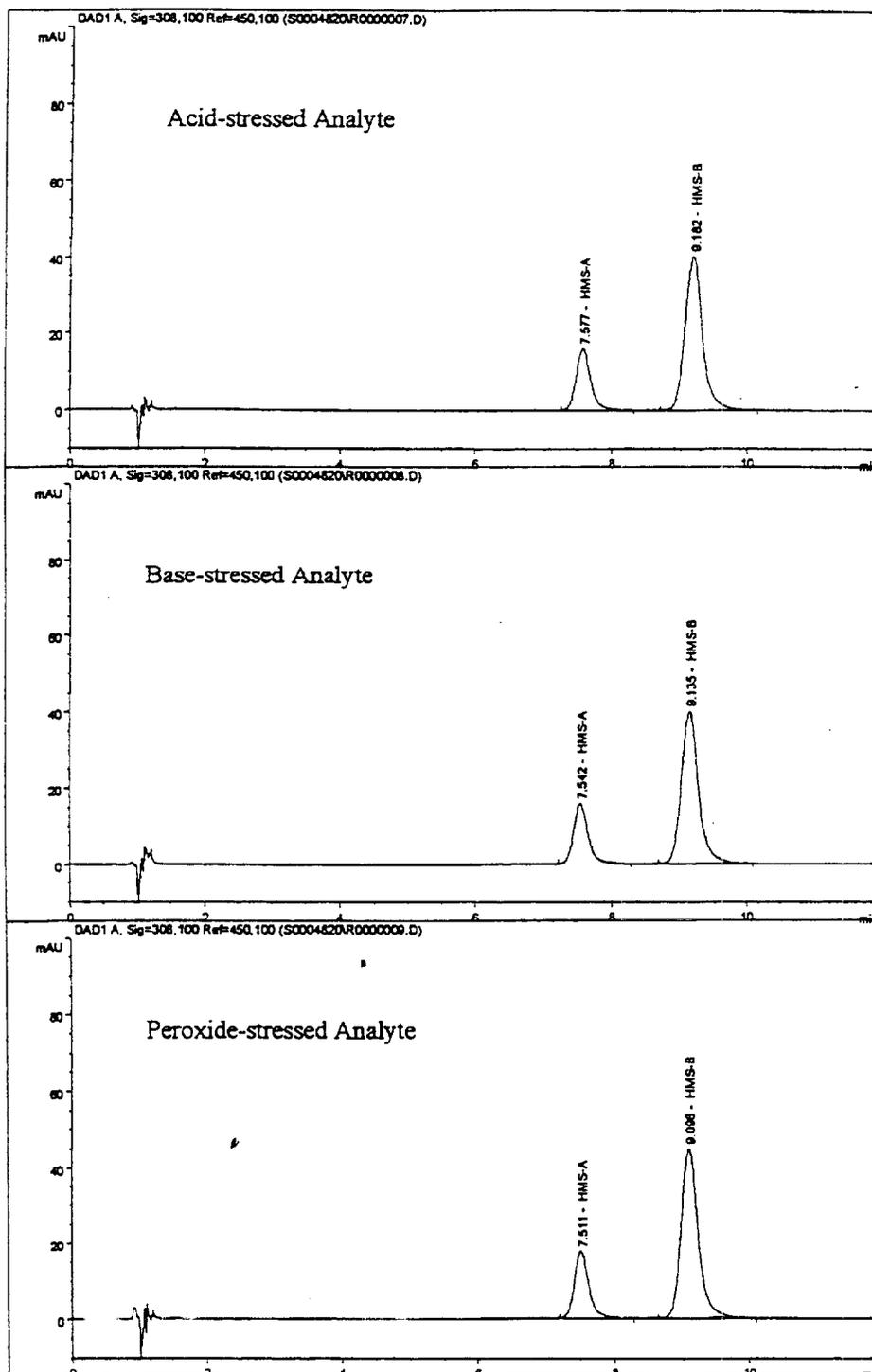


Figure 14. Chemically Stressed Analyte

Salicylic acid is a potential degradant of homomenthyl salicylate. A solution of salicylic acid and a solution of HMS spiked with salicylic acid were prepared and analyzed according to the analytical method in APPENDIX I (Figure 15). The chromatograms were reviewed for potential interference and the retention times of the degradant and the analyte were recorded. Table 10 contains the retention time data.

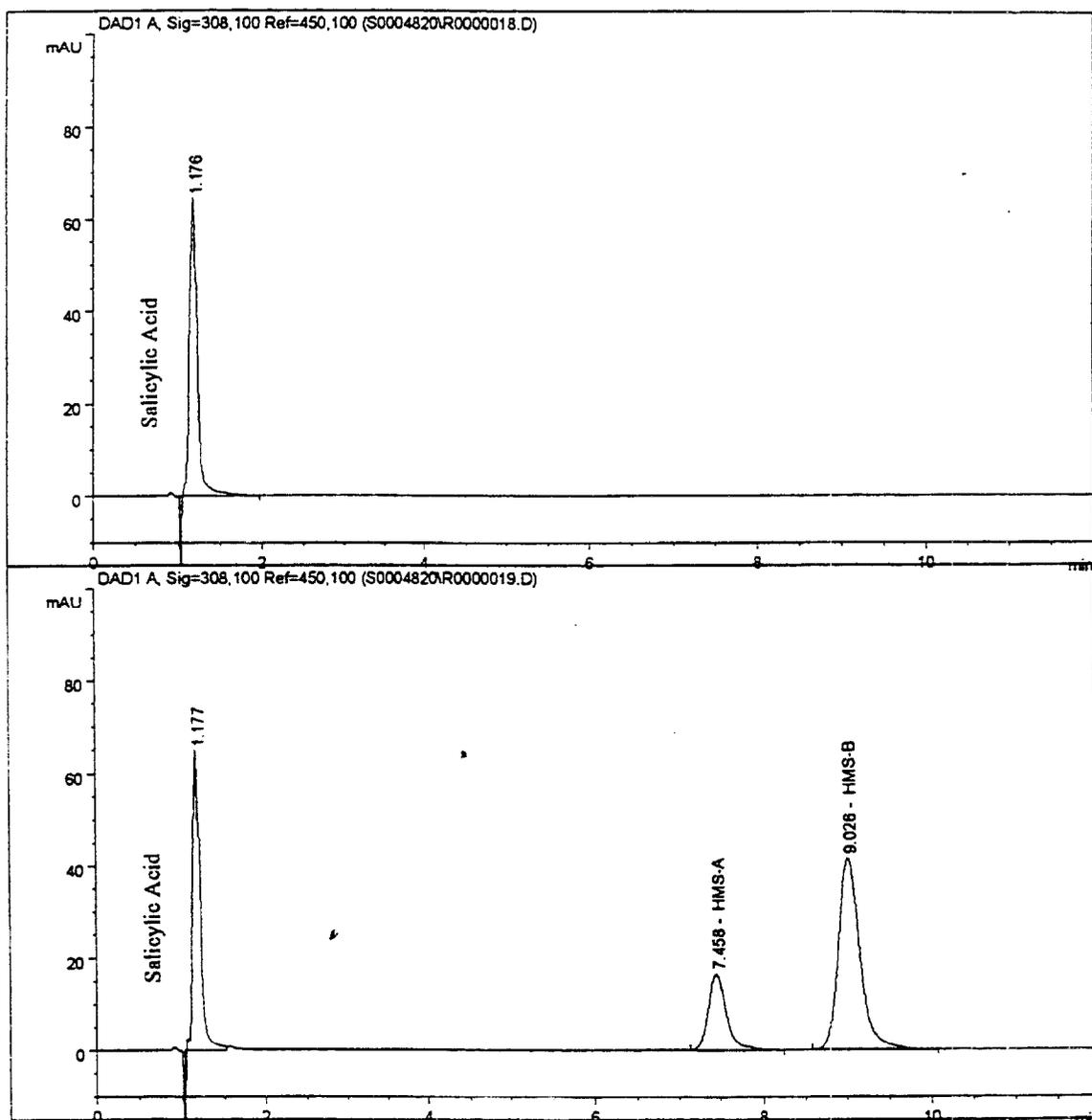


Figure 15. Salicylate Acid & HMS Spiked with Salicylate Acid

Table 10. Retention Times of Salicylic Acid & HMS

	Salicylic Acid	HMS-A	HMS-B
Salicylic Acid Solution	1.176	N/A	N/A
Salicylic Acid & HMS Solution	1.177	7.458	9.026

Acceptance Criteria:

- The analyte peaks in each chromatogram are pure by photodiode array analysis and any excipient, degradant or impurity peaks are resolved from the analyte peaks.

Conclusion:

The analyte and product purity factors are all greater than 990, indicating no interference. See Table 9. The degradant peak is resolved from the analyte. See Figure 15. The specificity data meets the acceptance criteria.

IV. CONCLUSION

The analytical method for the analysis of Homomenthyl Salicylate in 8% Homomenthyl Salicylate Standard Lotion, RB# 524-103 is suitable and valid.

ATTACHMENT 1

Sunscreen SPF 4 – Homomenthyl Salicylate
Assay (% w/w)

A. Reagents:

1. Acetic Acid, glacial, ACS grade
2. Isopropanol, HPLC grade
3. Methanol, HPLC grade
4. Homomenthyl Salicylate, Reference Standard

B. Instrumentation:

Equilibrate a suitable liquid chromatograph to the following or equivalent conditions:

Column : Ultrasphere ODS 150 x 4.6 mm (5 μ) or
Ultrasphere ODS 250 x 4.6 mm (5 μ)
Mobile Phase : 85:15:0.5 Methanol:Water:Acetic Acid
Flow Rate : 1.5 mL/min.
Temperature : Ambient
Detector : UV Spectrophotometer @ 308 nm
Attenuation : As needed
Injection Amount : 10 μ L

C. Mobile Phase Preparation

Mix 850 mL methanol, 150 mL of water and 5.0 mL of glacial acetic acid.

D. Standard Preparation:

1. Accurately weigh about 0.50 g of Homomenthyl Salicylate (HMS) Reference Standard into a 250-mL volumetric flask. Dissolve and dilute to volume with isopropanol. Mix well.
2. Accurately pipet 20.0 mL of the HMS stock solution (C.1.) into a 100-mL volumetric flask. Dilute to volume with isopropanol and mix well. This is the Standard Preparation.

E. Sample Preparation:

1. Accurately weigh approximately 2.0 g of sample into a 100-mL volumetric flask.

2. Add approximately 75 mL of isopropanol and heat with swirling until the sample is evenly dispersed.
3. Cool to room temperature and dilute to volume with isopropanol. Mix well.
4. Accurately pipet 25.0 mL of the sample solution (D.3.) into a 100-mL volumetric flask and dilute to volume with isopropanol. Mix well.

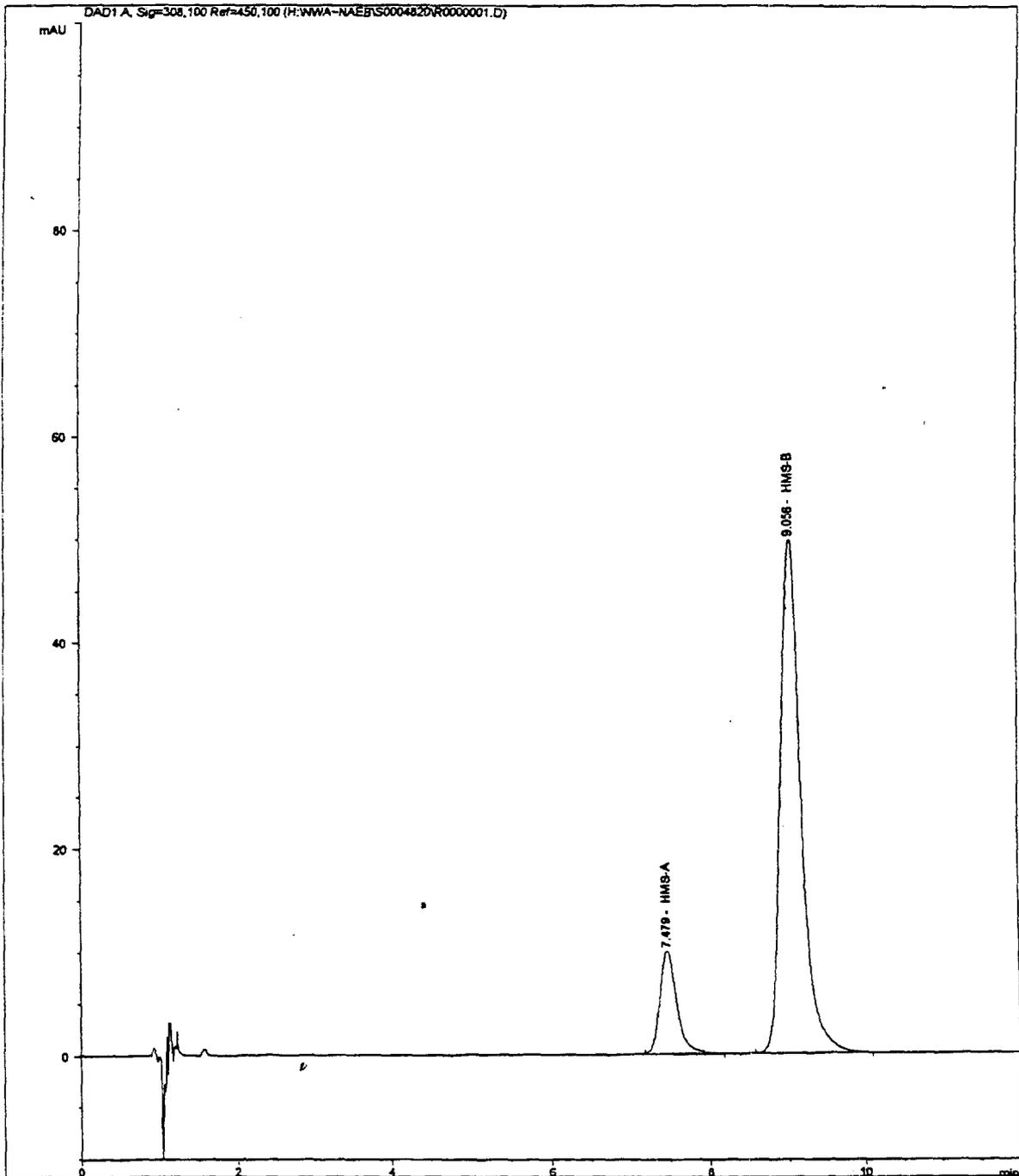
F. System Suitability:

An HPLC equilibrated to the above conditions would be considered suitable. This system would insure that three replicate injections of the Standard Preparation would yield a relative standard deviation of not more than 2.0% calculated on peak areas for HMS. Should a system fail to meet this criterion, adjusting the mobile phase or replacing the column may be necessary to obtain suitable chromatography.

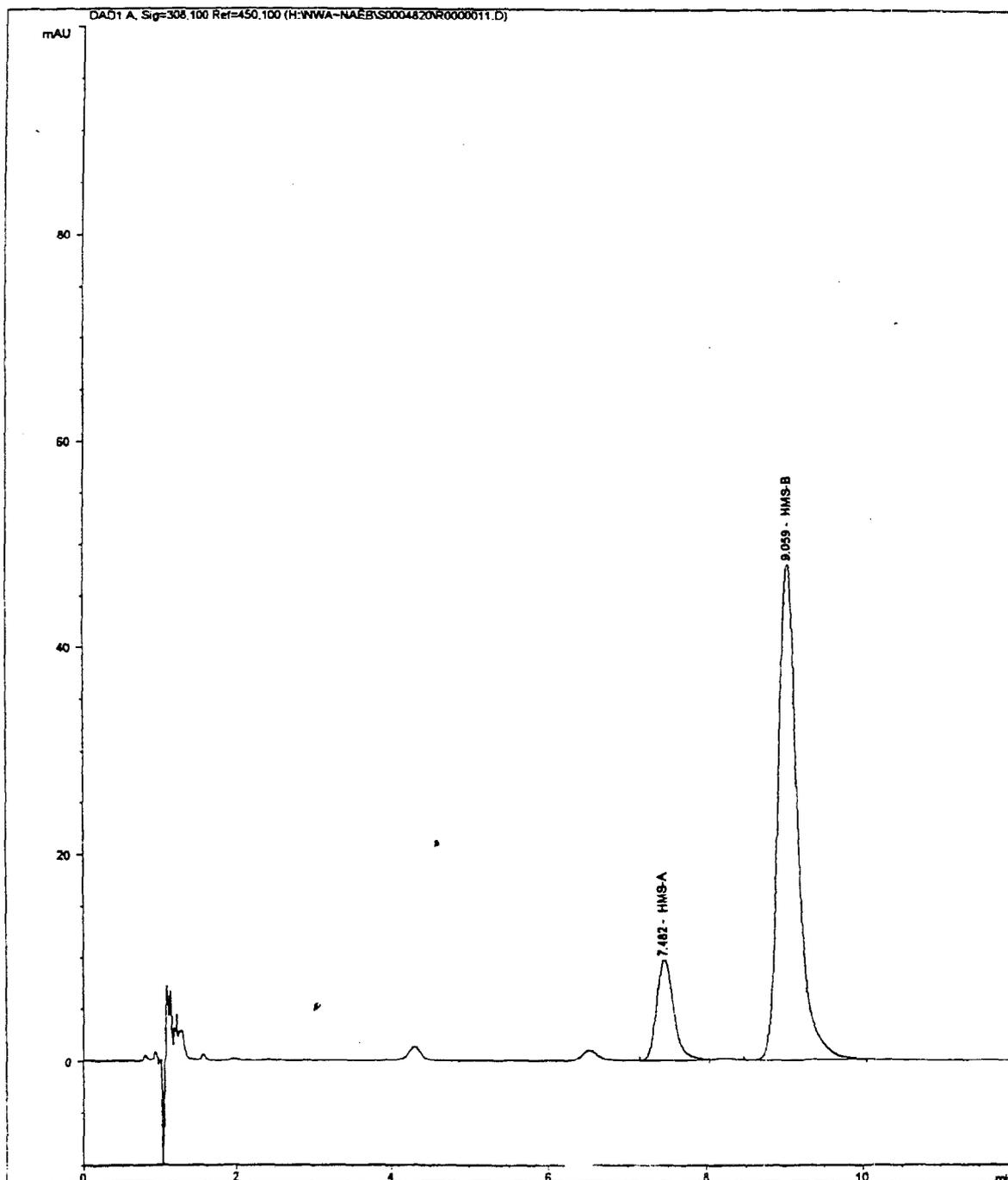
G. Analysis:

1. Inject 10 μ L of the Standard Preparation in triplicate collecting data for about 15 minutes or until both HMS peaks have completely eluted (two isomers). Determine if the system meets the suitability criteria as established above.
2. Similarly inject 10 μ L of each Sample Preparation.
3. Sum the peak areas of the two HMS isomers for each injection and calculate the HMS content in the sample as follows:

$$\frac{(\text{Total HMS Peak Area for Sample})(\text{Std. Wt. g})(32)}{(\text{Avg. Total HMS Peak Area for Standard})(\text{Smp. Wt. g})} = \text{HMS \% (w/w)}$$



Typical Standard Chromatogram



Typical Sample Chromatogram

**METHOD VALIDATION SECTION
FOR 8% HMS STANDARD LOTION**

A. Samples for Method Validation

The following samples are available pursuant to 21 CFR 314.50(e)(1)(i) and will be provided upon request.

Samples for Method Validation may be obtained by contacting:

**Dr. C. Rainey
Director, Analytical Research and Development
Schering-Plough HealthCare Products
Memphis, TN 38151
(901)320-2496**

Four identical separately packaged subdivisions each containing the samples listed below will be provided:

- One bottle containing 50 grams of 8% HMS Standard Lotion, Lot Number P58018
- One bottle containing 50 grams of 8% HMS Standard Lotion PLACEBO without HMS, Lot Number P58012
- One bottle containing 15 grams of Homomenthyl Salicylate drug substance, Lot Number ER980237, used in the manufacture of 8% HMS Standard Lotion, Lot Number P58018
- One bottle containing 2 grams of Homomenthyl Salicylate Reference Material, RS025845

B. Certificates of Results

The following certificates of analysis are provided.

- a. 8% HMS Standard Lotion, Lot Number P58018
- b. Homomenthyl Salicylate drug substance, Lot Number ER980237
- c. Homomenthyl Salicylate Reference Material, RS025845