EXHIBIT 6

DECLARATION OF THOMAS W. STRICKLAND

I, Thomas W. Strickland, solemnly declare as follows:

1. I make this declaration in support of the oppositions against EP 209539 entitled "Homogeneous Erythropoietin" to Genetics Institute.

2. Since April 1984, I have been employed by Amgen Inc., located in Thousand Oaks, California, and I currently hold the position of Research Scientist. I received my doctorate degree (Ph.D.) in Biochemistry from Vanderbilt University in 1981 and did my thesis on glycoprotein hormones. From February 1981 to March 1984, I held the position of Assistant Research Biological Chemist, Department of Biological Chemistry, University of California, Los Angeles (UCLA). My research activities at UCLA included studies on biosynthesis and protein folding of glycoprotein hormones and the chemical modification, isolation and receptor cross-linking of glycoprotein hormones. A copy of my curriculum vitae is attached as Exhibit A.

3. Since joining Amgen in 1984, one of my responsibilities has been to work on the purification of recombinant erythropoietin ("rEPO"). During my years at Amgen, I have also been extensively involved in the analyses of erythropoietin including both recombinant and urinary-derived EPO.

4. I have reviewed the disclosure of EP 209539, and I am familiar with its contents. I was involved in the litigation in the United States between Amgen and Genetics Institute in which the U.S. counterpart to EP 209539 (U.S. Patent No. 4,677,195) was held invalid.

5. I am also familiar with the disclosure of EP 148605 entitled "Production of Erythropoietin" to Kirin-Amgen. In particular, Example 10 of that patent discloses the production of recombinant EPO using Chinese Hamster Ovary (CHO) cells which have been transfected with a plasmid containing the DNA sequence encoding human EPO. Example 10 provides a description of this production process. Amgen's past and current commercial process for producing erythropoietin follows the procedures of Example 10 in the expression of rEPO from CHO cells. Example 10 discloses the use of serum free cell culture medium in order to facilitate the

purification of the rEPO. See EP 148605, page 28, lines 20-24. Example 10 further indicates that "Mammalian cell expression products may be readily recovered in substantially purified form from culture media using HPLC (C4) employing an ethanol gradient, preferably at pH7." EP 148605, page 29, lines 4-5. A C4 HPLC column using an ethanol gradient is a reverse phase HPLC column. EP 148605 also discloses that other conventional purification processes may be used. See, page 9, lines 1-3, EP 148605.

6. In order to demonstrate the viability of the specific disclosure of Example 10 of EP 148605, reverse phase HPLC was used to purify rEPO directly from cell culture media in which the rEPO had been expressed from CHO cells as described in Example 10. The results show that by following the disclosure of Example 10 homogeneous erythropoietin is obtained that meets all the requirements of claim 2 of EP 209539, *i.e.*, (a) movement as a single peak on a reverse phase-HPLC; (b) a molecular weight of about 34,000 daltons on SDS-PAGE; and (c) a specific activity of about 160,000 IU per absorbance at 280 nm as indicated by *in vivo* assay.

7. A 100 ml sample of cell conditioned media was obtained following the disclosure of Example 10 of EP 148605 which describes the expression of rEPO from CHO cells in serum free cell culture media. The conditioned media sample was purified utilizing a single-step reverse phase column employing an ethanol gradient at pH 7 as described in Example 10 of EP 148605. As the first step in the HPLC procedure, the sample of conditioned media was filtered through a 0.45 micron cellulose nitrate filter prior to running the sample on the reverse phase HPLC column. The Ethanol/Tris gradient buffers were also filtered through a 0.45 micron Nylon-66 solvent resistant filter as required prior to use on the column. Following the loading of the 100 ml conditioned media sample to the 1 X 25 cm Vydac C4 column, the column was developed with a gradient from 20% Ethanol/10 mM Tris-HCl, pH 7 to 80% Ethanol/10 mM Tris-HCl, pH 7 at a rate of approximately 0.13% Ethanol per minute at a flow rate of 1 ml/minute. The absorbance profile at 280 nm of the purification chromatogram is attached as Exhibit B. Fractions of 3 ml each were collected into 12 ml of water in order to dilute the ethanol. In order to remove the ethanol and the water, the fractions were dried overnight using a Speedvac (vacuum device in conjunction with low speed centrifugation) and resuspended in water the next day.

8. The EPO containing fractions indicated by the bar in Exhibit B were pooled and then assayed by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) to indicate an apparent molecular weight with respect to BioRad broad range molecular weight standards. The resulting gels, one run with reduction of the protein disulfide bonds and one run in the absence of reductant, are shown in Exhibit C. The EPO containing fraction (indicated as "Test Sample" on the gels) falls between the markers for 31 kDa and 45 kDa and migrates in the same region as Amgen's commercial rEPO product. In my opinion, the Test Sample migrates on SDS-PAGE within the range encompassed by the criterion "about 34,000 daltons" as indicated in Claim 2 of EP 209539.

9. In order to provide a solvent control for subsequent analytical HPLC analysis, a blank gradient of Ethanol/Tris buffer was run on the 1 X 25 cm reverse phase column. This run was identical to the purification described above except that no sample was loaded. Fractions collected from this gradient were treated as for the purification run (*i.e.* diluted with water, dried in Speedvac, and redissolved in water). The blank run fractions corresponding to the fractions pooled from the purification run were themselves pooled in order to provide the appropriate solvent blank. These pooled fractions from the blank run were then analyzed on the analytical RP-HPLC under the conditions described in EP 209539 to provide an analysis of the blank carrier.

10. The sample of EPO containing fractions was then analyzed using a reverse phase HPLC column under the solvent and gradient conditions disclosed in EP 209539 using a 0.46 X 25 cm Vydac C4 column in order to demonstrate that the purified fraction is homogeneous EPO and moves as a single peak on reverse phase HPLC as required by Claim 2 of EP 209539. The resulting absorbance at 280 nm of the analytical chromatogram is shown in Exhibit D and clearly shows a single peak. As can be seen, the minor peaks before and after the EPO peak are due entirely to the blank carrier.

11. Finally, the purified EPO sample was assayed by the exhypoxic polycythemic mouse *in vivo* bioassay. Assay samples were prepared by first diluting the purified sample to the appropriate extent and then preparing a two-fold dilution series (yielding a total of four sample dilutions). Each dilution was then injected into five mice. The samples were assayed by Dr. Peter Dukes' laboratory at Children's

Hospital of Los Angeles in the exhypoxic polycythemic mouse bioassay essentially according to the method of Cotes and Bangham (*Nature*, **191**:1065-1067 (1961)). Dr. Dukes has been performing this assay for Amgen since the early 1980's. The purified sample was assayed in four successive assays and the results were as follows:

Assay	U/A_{280}
1	147,600
2	181,900
3	146,700
4	152,200
Average	157,100 ± 16,700

12. I have read the submission of Professor Kula and I agree that the culture media must first be freed from particulate matter prior to loading the sample on a HPLC column. The need to filter any sample prior to injecting it onto an HPLC column has been recognized as a required preliminary step since the early use of HPLC. Even the gradient buffers used in the HPLC procedure must be filtered prior to use. Consequently, this filtering step is simply a procedure to be performed in operating the HPLC column.

13. The results discussed above show that a homogeneous EPO having the properties as disclosed and claimed by Genetics Institute was obtained following the procedures of Example 10 of EP 148605.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Date: May 19, 1994

Thomas W. Strickland

Thomas W. Strickland

EXHIBIT A

Curriculum Vitae

THOMAS WAYNE STRICKLAND

Date of Birth: 09/14/54

Place of Birth: Pensacola, Florida

Home Address: 13450 E. Cedarpine
Moorpark, California
(805) 523-8740

Office Address: Amgen Inc., Amgen Center
Thousand Oaks, California 91320
(805) 499-5725 (x3088)

Education:

University of West Florida	B.S., 1976 (Biology)
University of West Florida	B.S., 1976 (Chemistry)
Vanderbilt University	Ph.D., 1981 (Biochemistry)

Ph.D. Thesis:

Contribution of Subunits to Glycoprotein Hormone Function: A Study Utilizing Hybrids

Work Experience:

April 1984 - present
Research Scientist, Protein Chemistry Department, Amgen Inc. Research principally involved in the purification and characterization of urinary and recombinant human erythropoietin

February 1981 - March 1984
Assistant Research Biological Chemist, Department of Biological Chemistry, University of California Los Angeles. Research included studies on biosynthesis and protein folding of glycoprotein hormones; chemical modification, isolation, and receptor cross-linking of glycoprotein hormones

Honors and Offices:

Pensacola Junior College Faculty Scholarship Award (1974)
Foundation Scholarship, University of West Florida (1974-76)
National Science Foundation Undergraduate Research Fellowship (1975)
Monsanto Award for Chemistry (1976)
Graduate Summa Cum Laude, University of West Florida
National Institutes of Health Predoctoral Traineeship (1976-77)
National Science Foundation Predoctoral Fellowship (1977-1980)
Harold Sterling Vanderbilt Graduate Scholarship (1976-1980)

Bibliography

Papers:

1. Metabolism of vinyl chloride: destruction of the heme of highly purified cytochrome P-450 by a metabolite. F. Peter Guengerich and Thomas W. Strickland. *Molecular Pharmacology 13*, 993-1004 (1977).

2. Gonadotropin-testicular receptor interactions and subunit-subunit interactions. T.W. Strickland, T.D. Noland and D. Puett. In *Functional Correlates of Hormone Receptors in Reproduction* (eds. V.B. Mahesh and T. Muldoon), Elsevier Publ. Co. (1981).

3. Contribution of subunits to the function of luteinizing hormone/human chorionic gonadotropin recombinants. Thomas W. Strickland and David Puett, *Endocrinology 109*, 1933-1942 (1981).

4. The kinetic and equilibrium parameters of subunit association and gonadotropin dissociation. Thomas W. Strickland and David Puett. *J. Biol. Chem. 257*, 2954-2960 (1982).

5. Alpha-subunit conformation in glycoprotein hormones and recombinants as assessed by specific antisera. Thomas W. Strickland and David Puett. *Endocrinology 111*, 95-100 (1982).

6. Circular dichroism of gonadotropin recombinants. Thomas W. Strickland and David Puett. *Int. J. Peptide Protein Res. 21*, 374-380 (1983).

7. The alpha-subunit of pituitary glycoprotein hormones. Formation of three-dimensional structure during cell-free biosynthesis. Thomas W. Strickland and John G. Pierce. *J Biol. Chem. 258*, 5927-5932 (1983).

8. Thyrotropin (TSH) purification and characterization. John G. Pierce, Thomas F. Parsons and Thomas W. Strickland. "Handbook on Receptor Research", S. Aloj, ed. (1983).

9. Disassembly and assembly of glycoprotein hormones. Thomas F. Parsons, Thomas W. Strickland and John G. Pierce. *Methods in Enzymology 109*, 736-749 (1985).

10. Rapid and easy separation of the subunits of bovine and human glycoprotein hormones using high performance liquid chromatography. Thomas F. Parsons, Thomas W. Strickland and John G. Pierce. *Endocrinology 114*, 2223-2227 (1984).

11. Structure of LH and hCG. Thomas W. Strickland, Thomas F. Parsons and John G. Pierce. "CRC Critical Review: Gonadotropin Receptors and Gonadotropin Action", M. Ascoli, ed., CRC Press, Inc. (1985).

12. Glycosylation of ovine prolactin during cell-free biosynthesis. T.W. Strickland and J.G. Pierce. *Endocrinology 116*, 1295-1298 (1985).

13. Tyrosine residues of bone tyrotropin - accessibility to iodination in the intact hormone and isolated subunits. T.W. Strickland, J.F. Williams and J.G. Pierce. *Intl. J. Peptide Protein Res. 24*, 328-336. (1984)

14. Erythropoietin - gene cloning, protein-structure, and biological properties. J.K. Browne, A.M. Cohen, J.C. Egrie, P.H. Lai, T. Strickland, E. Watson and N. Stebbing. Cold Spring Harbor Symposia on Quantitative Biology *51*, 693-701 (1986).

15. The common alpha-subunit of bovine glycoprotein hormones - limited formation of native structure by the totally nonglycosylated polypeptide chain. T.W. Strickland, A.R. Thomason, J.H. Nilson and J.G. Pierce. *Journal of Cellular Biochemistry 29*, 225-237 (1985).

16. The beta-subunit of thyrotropin can be cross-linked to its receptor by disuccinimidyl suberate. P.R. Buckland, T.W. Strickland and B.R. Smith. *Biochemical Society Transactions 13*, 942-943 (1985).

17. The beta-subunits of glycoprotein hormones - formation of three-dimensional structure during cell-free biosynthesis of lutropin-beta. T.W. Strickland and J.G. Pierce. *Journal of Biological Chemistry 260*, 5816-5819 (1985).

18. TSH crosslinks to the TSH receptor through the beta-subunit. P.R. Buckland, T.W. Strickland, J.G. Pierce and B.R. Smith. *Endocrinology 116*, 2122-2124 (1985).

19. Characterization of recombinant human erythropoietin produced in Chinese hamster ovary cells. J.M. Davis, T. Arakawa, T.W. Strickland and D.A. Yphantis. *Biochemistry 26*, 2633-2638 (1987).

20. Recombinant DNA-derived bovine growth hormone from *Escherichia coli*. I. Demonstration that the hormone is expressed in reduced form, and isolation of the hormone in oxidized, native form. K.E. Langley, T.F. Berg, T.W. Strickland, D.M. Fenton, T.C. Boone and J. Wypych. *European Journal of Biochemistry 163*, 213-224 (1986).

21. Characterization and biological effects of recombinant human erythropoietin. J.C. Egrie, T.W. Strickland, J. Lane, K. Aoki, A.M. Cohen, R. Smalling, G. Trail, F.K. Lin, J.K. Browne and D.K. Hines. *Immunobiology 172*, 213-224 (1986).

22. Megakaryocyte colony stimulating activity of recombinant human and monkey erythropoietin. P.O. Dukes, J.C. Egrie, T.W. Strickland, J.K. Browne and F.K. Lin. *Prog. Clin. Biol. Res. 215*, 105-109 (1986).

23. Comparative studies of natural and recombinant erythropoietin. D. Vapnek, J.C. Egrie, J.K. Browne, P. Lai, F.K. Lin, T. Arakawa and T.W. Strickland. *Banbury Reports 29* (Therapeutic Peptides and Proteins), 241-256 (1988).

AF 51187

24. Relationship between sugar chain structure and biological activity of recombinant human erythropoietin produced in Chinese hamster ovary cells. M. Takeuchi, N. Inoue, T.W. Strickland, M. Kubota, M. Wada, R. Shimizu, S. Hoshi, H. Kozutsumi, S. Takasaki and A. Kobata. *Proc. Natl. Acad. Sci. USA 20*, 7819-7822 (1989).

25. The effect of carbohydrate on the structure and stability of erythropoietin. L.O. Narhi, T. Arakawa, K.H. Aoki, R. Elmore, .F. Rohde, T. Boone and T.W. Strickland. *Journal of Biological Chemistry 266*, 23002-23026 (1991).

26. Densimetric determination of carbohydrate content in glycoproteins. C.G. Kolvenbach, K.E. Langley, T.W. Strickland, W.C. Kenney and T. Arakawa. *Journal of Biochemical and Biophysical Methods 23*, 295-300 (1991).

Abstracts:

1. Vinyl chloride mediated P-450 destruction. Thomas Strickland and F. Peter Guengerich. *Federation Proceedings 36* (3) 991 (1977).

2. Sedimentation velocity and sedimentation equilibrium of ovine lutropin alpha. Thomas Strickland, L.A. Holladay, Mario Ascoli and David Puett. *Federation Proceedings 37* (5), 1712 (1978).

3. Gonadotropin recombinants: A correlation of biological potency with alpha-subunit conformation. Thomas W. Strickland and David Puett. Presented at the 62nd Annual Meeting of the Endocrine Society (Abstract No. 11). Washington, D.C. (1980).

4. Kinetic and equilibrium parameters of gonadotropin assembly and disassembly. Thomas W. Strickland and David Puett. Presented at the 63rd Annual Meeting of the Endocrine Society (Abstract No. 805) Cincinnati, OH (1981).

5. Folding of the alpha subunit of lutropin after *in vitro* biosynthesis. Thomas W. Strickland, Joan M. Goverman and John G. Pierce. Presented at the 64th Annual Meeting of the Endocrine Society (Abstract No. 198) San Francisco, CA (1982).

6. TSH crosslinks to the TSH receptor through the beta-subunit. P.R. Buckland, T.W. Strickland, J.G. Pierce, R.D. Howells and C.R. Rickards. *Annales D. Endocrinologie 45*, 52 (1984).

7. Biophysical characterization of recombinant human erythropoietin produced in Chinese hamster ovary cells. J.M. Davis, T.W. Strickland, D.A. Yphantis and T. Arakawa. *Biophysical Journal 49*, 499 (1986).

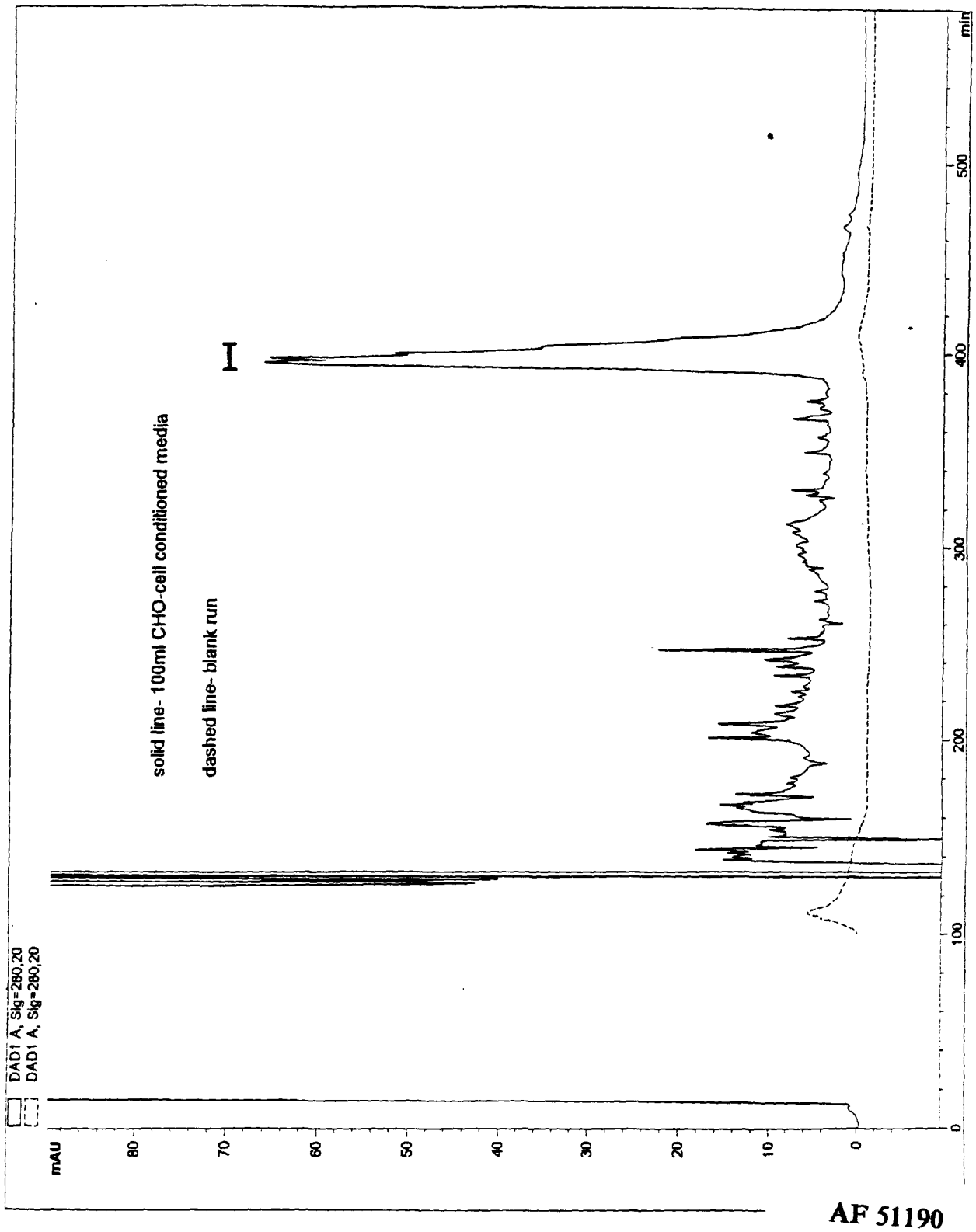
8. Characterization of pure human recombinant erythropoietin. J.C. Egrie, T.W. Strickland, J. Lane, K. Aoki, R. Smalling, G. Trail, F.K. Lin, J.K. Browne, D.K. Hines and A.M. Cohen. *Experimental Hematology 13*, 458 (1985).

9. *In vitro* and *in vivo* megakaryocytopoietic effects of recombinant erythropoietin. P.P. Dukes, J.C. Egrie, T.W. Strickland, J.K. Browne and F.K. Lin. *Experimental Hematology 14*, 469 (1986).

10. Structural characterization of recombinant DNA-derived human erythropoietin. C.G. Parker, T.W. Strickland, K.H. Aoki and P.H. Lai. *Federation Proceedings 45*, 1721 (1986).

11. Characterization of recombinant monkey erythropoietin. R.H. Elmore, K.H. Aoki, T.W. Strickland, F.K. Lin and M.F. Rohde. Presented at the Protein Society Annual Meeting, July, 1989.

EXHIBIT B

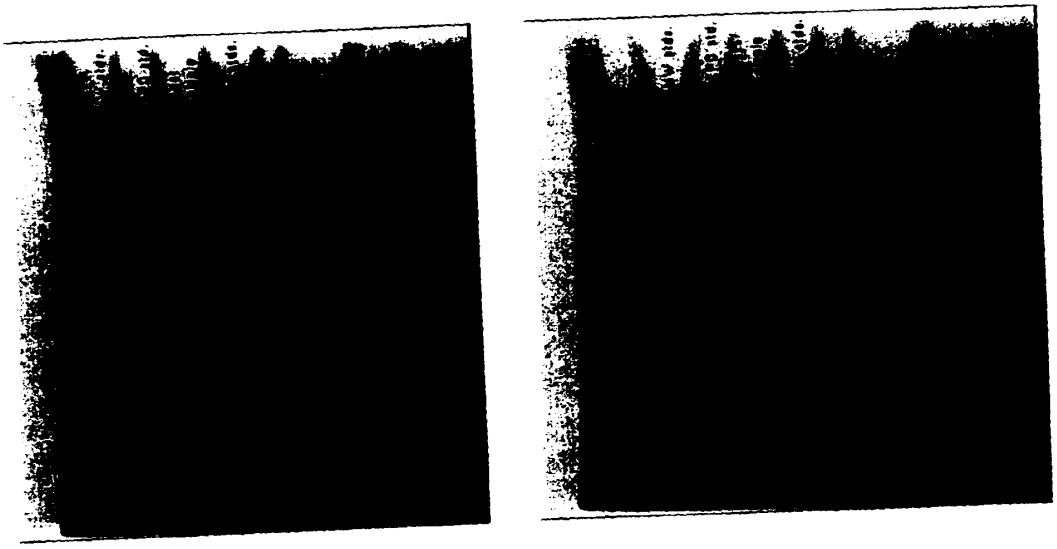


AF 51190

EXHIBIT C

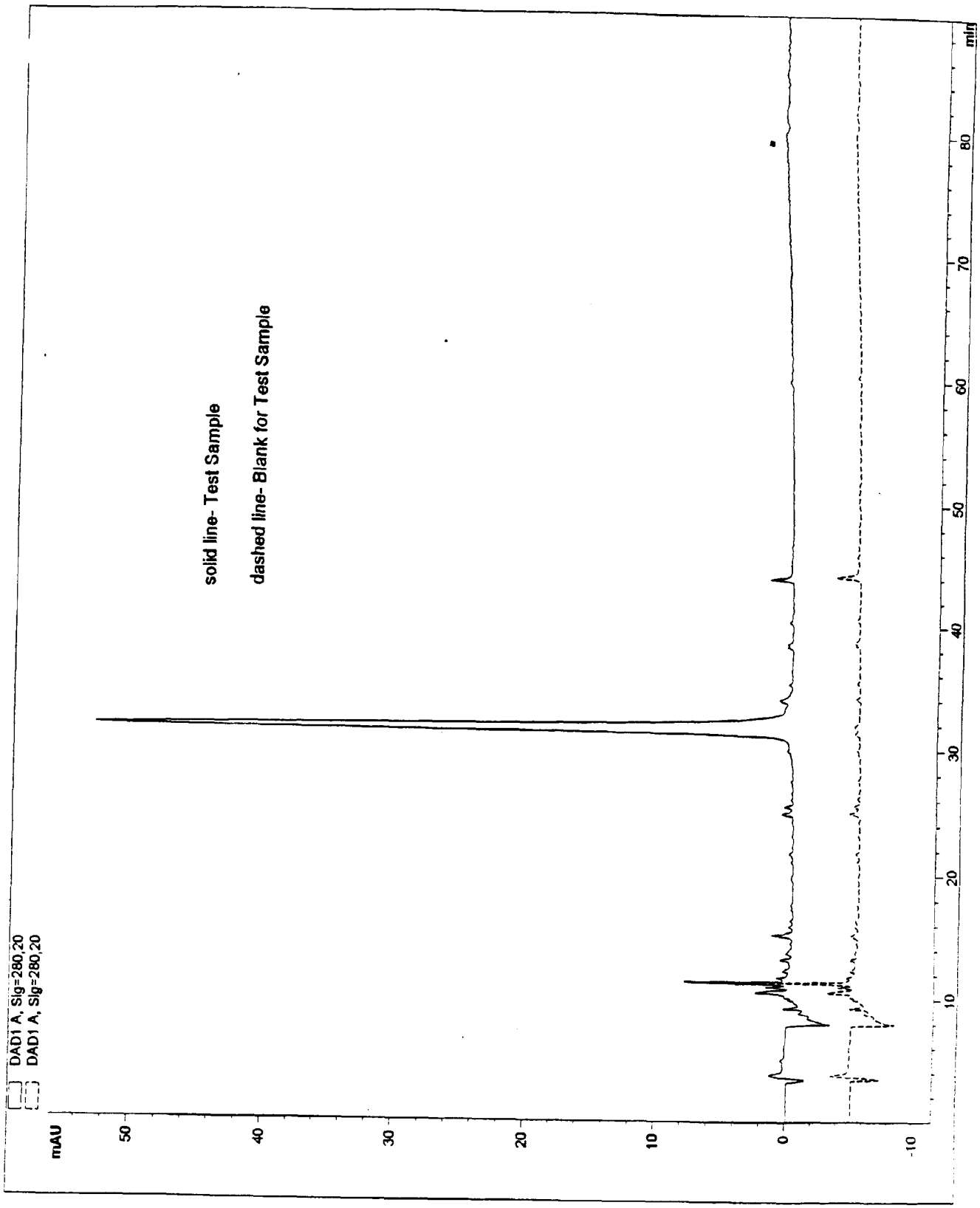
Non-reducing

Reducing



AF 51191

EXHIBIT D



AF 51192