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Poster Presentation

Purification of Synthetic Peptide M by Reverse Phase HPLC

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PURIFICATION OF SYNTHETIC PEPTIDE M BY RP-HPLC

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ABSTRACT

AN OCTADECAPEPTIDE, PEPTIDE M, THE EPITOPE OF A RETINAL PROTEIN THAT INDUCES EXPERIMENTAL AUTOIMMUNE UVEITIS, WAS SYNTHESIZED AND PURIFIED BY PREPARATIVE REVERSED-PHASE CHROMATOGRAPHY. THE FLOW RATE AND GRADIENT CONDITIONS FOR MAXIMUM SEPARATION OF IMPURITIES WERE DETERMINED ON A 3.9 MM X 30 CM COLUMN OF DELTA PAK (15 UM SPHERICAL C18 BONDED SILICA WITH 300 A PORES). THE MAXIMUM AMOUNT OF PEPTIDE THAT WAS RESOLVED WITH THESE CONDITIONS WAS THEN EXPERIMENTALLY DETERMINED. USING A SCALE FACTOR DEPENDENT ON THE RELATIVE SQUARES OF THE COLUMN DIAMETERS, THE FLOW AND AMOUNT LOADED WERE INCREASED 165 TIMES ON A 5.0 CM X 30 CM COLUMN OF THE SAME PACKING. THE SEPARATION WAS ACHIEVED. AMOUNTS OF 200 TO 300 MG WERE CHROMATOGRAPHED WITH REPRODUCIBLE RESULTS PROVIDING A YIELD OF 394 MG PURE PEPTIDE.

PEPTIDE M

Asp-Thr-Asn-Leu-Ala-Ser-Ser-Thr-Ile-Ile-Lys-Glu-Gly-Ile-Asp-

Arg-Thr-Val

EPITOPE SEQUENCE OF BOVINE RETINAL S-ANTIGEN

INDUCES EXPERIMENTAL AUTOIMMUNE UVEITIS

MANUAL SOLID PHASE SYNTHESIS

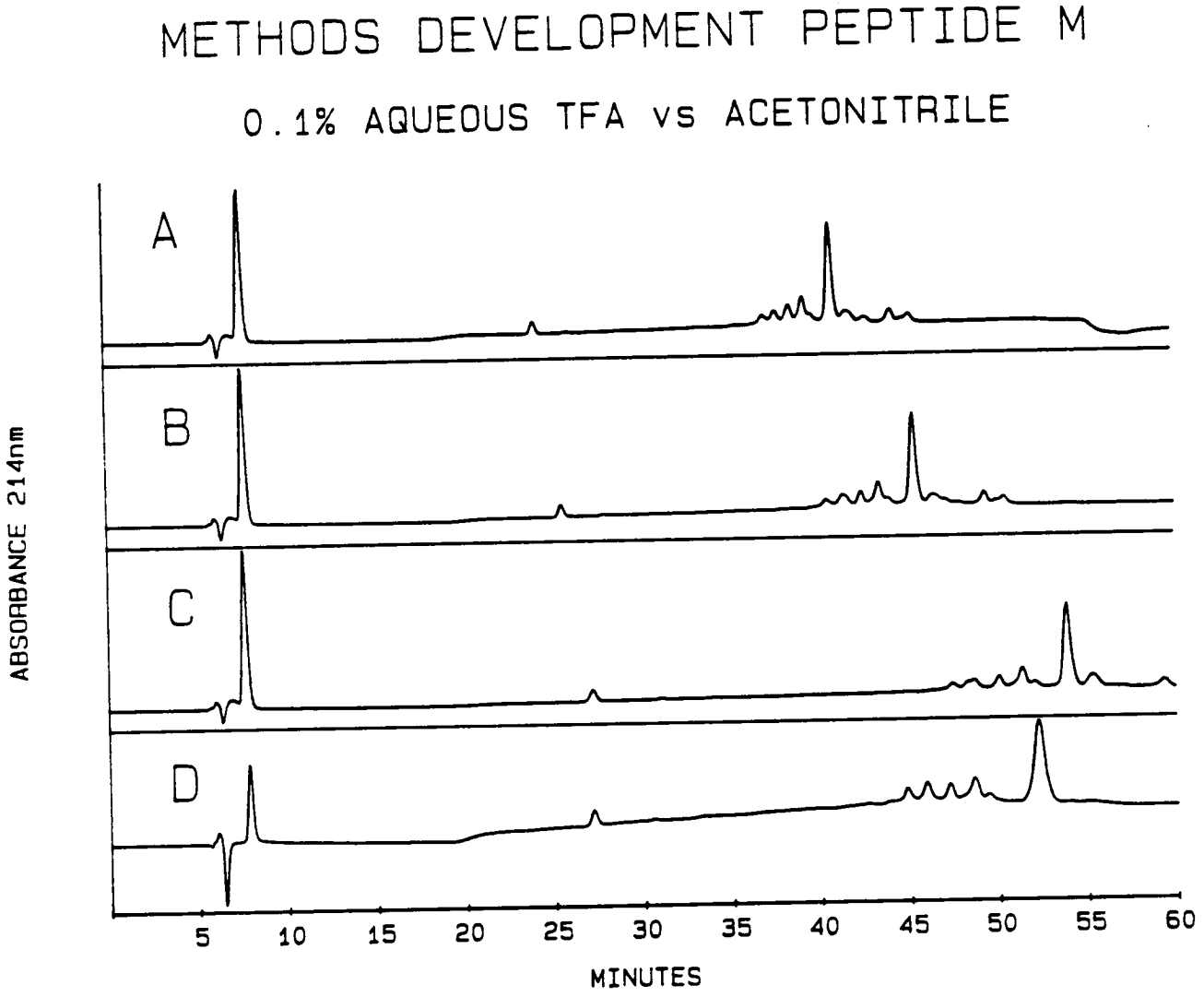


FIGURE 1. Optimization of chromatographic conditions for the purification of synthetic Peptide M on a 3.9 mm x 30 cm Delta Pak, 300 A, 15 u, C18 column. Buffer A was 0.1% aqueous TFA and Buffer B was acetonitrile with 0.1% TFA. The gradient conditions for each separation were the following: PANEL A, 0-50% B, 40 min, PANEL B, 0-40% B, 40 min, PANEL C, 0-30% B, 40 min, PANEL D, 0-25% B, 30 min. All the separations were run at a flow rate of 0.5 ml/min. Detection was at 214nm. The full scale absorbance of PANELS A, B and C was 0.7 and PANEL D 0.06 AUFS.

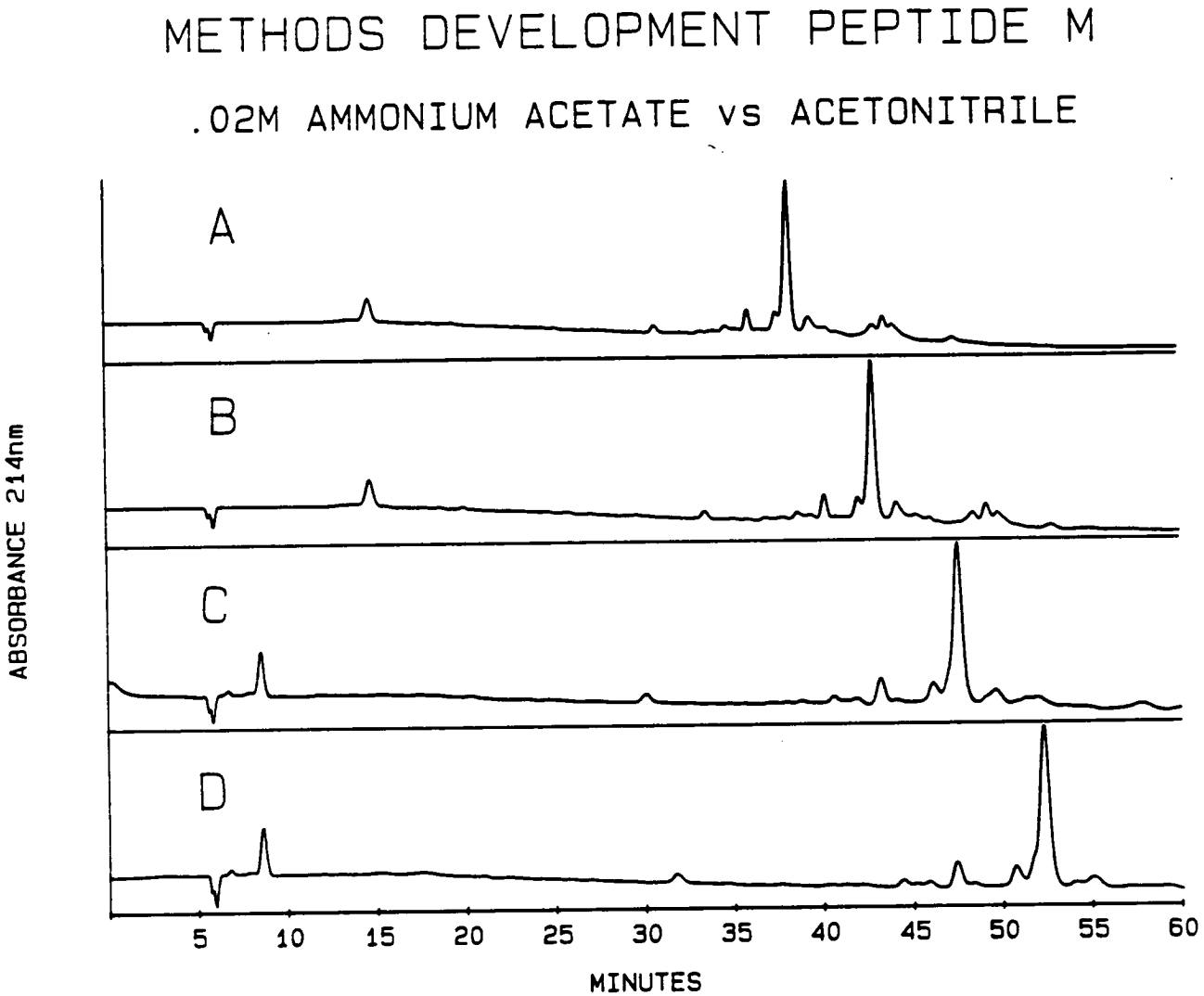


FIGURE 2. Optimization of chromatographic conditions for the purification of synthetic Peptide M on a 3.9 mm x 30 cm Delta Pak, 300 A, 15 u, C18 column. Buffer A was 0.02 M ammonium acetate, pH 6.8 and Buffer B was acetonitrile. The gradient conditions for each separation were the following: PANEL A, 0-30% B, 40 min, PANEL B, 0-25% B, 40 min, PANEL C, 0-20% B, 40min, PANEL D, 5-18% B, 40 min. All the separations were run at a flow rate of 0.5 ml/min. Detection was at 214nm. The full scale absorbance of PANELS A and B was 0.5 and PANELS C and D 0.3 AUFS.

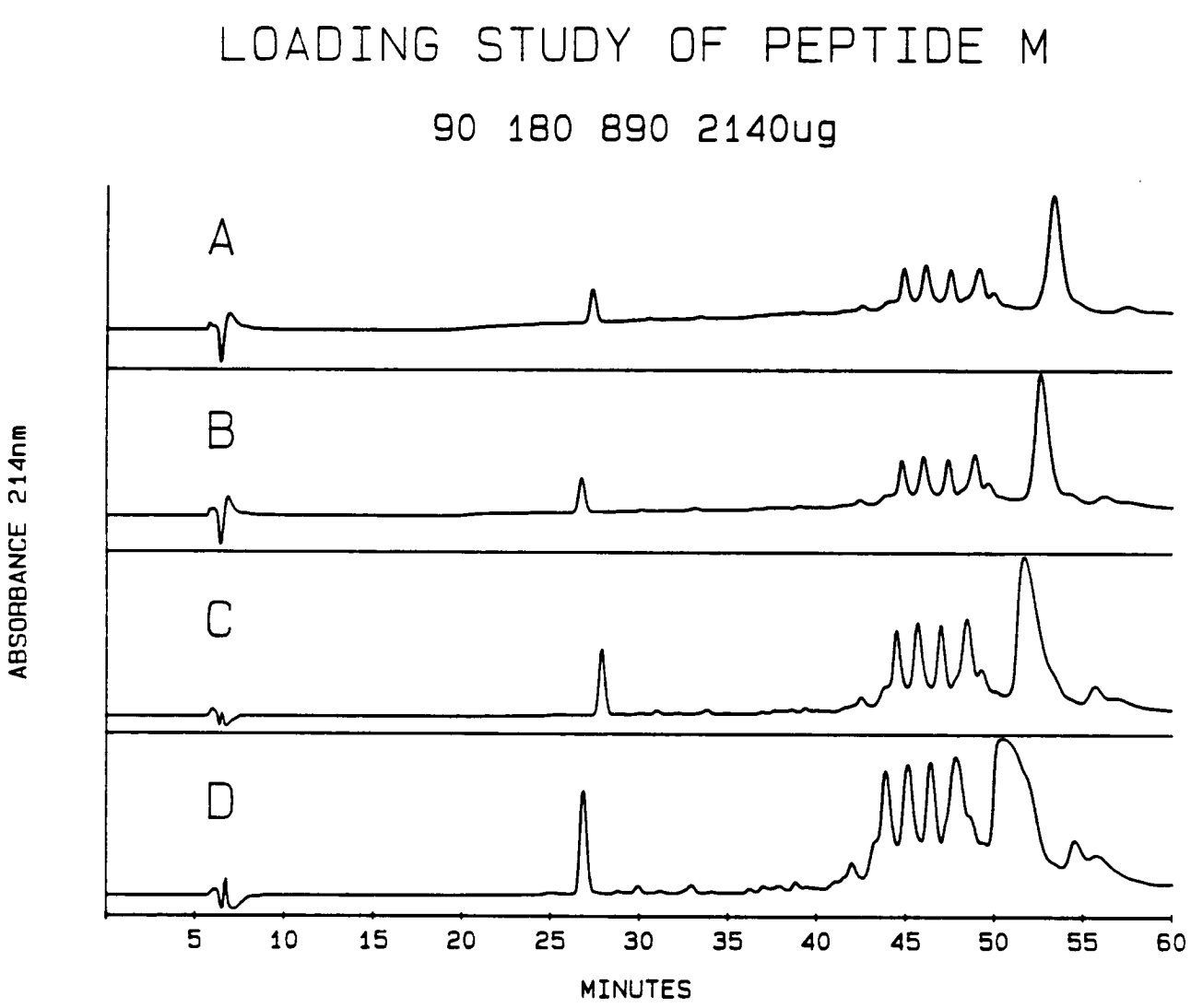
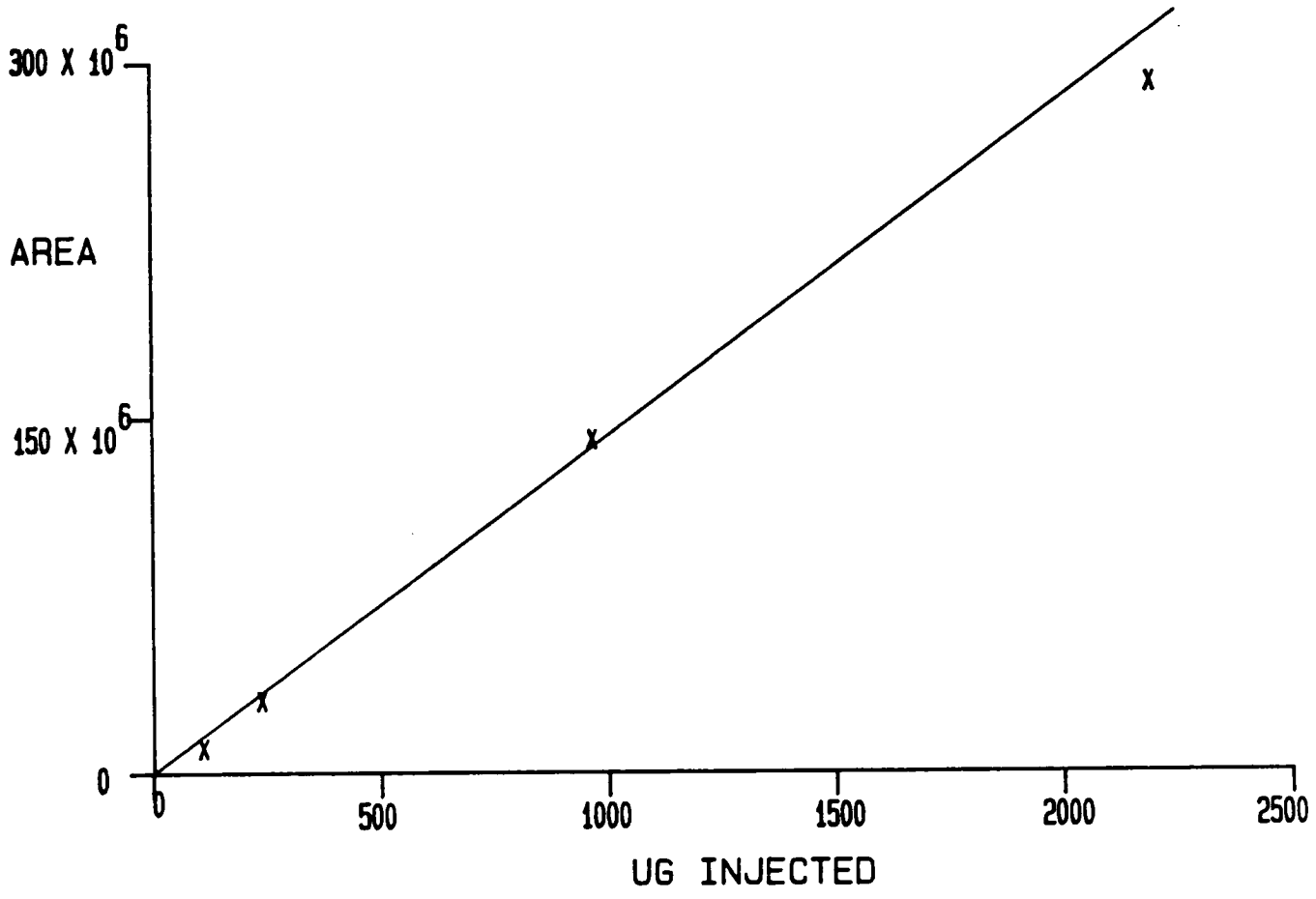


FIGURE 3. Determination of preparative sample amounts. Separations were performed on the 3.9 mm x 30 cm Delta Pak, 300 A, 15 u, column using a 30 minute linear gradient from 100% 0.1% aqueous TFA to 25% acetonitrile, 0.1% TFA / 75% aqueous 0.1% TFA at a flow rate of 0.5 ml/min. PANEL A shows the separation of 90 ug of crude sample PANEL B 180 ug, PANEL C 890 ug and PANEL D 2140 ug. Detection was at 214 nm. The full scale absorbance of PANEL A was 0.25, PANEL B 0.5, PANEL C 1.5 and PANEL D 2.0 AUFS.

MASS RECOVERY OF PEPTIDE M



DIRECT SCALE-UP BY CROSS SECTIONAL AREA - CONSTANT COLUMN LENGTH

COLUMN ID	SCALE FACTOR
3.9mm	1
7.8mm	4
19mm	24
30mm	60
50mm	165

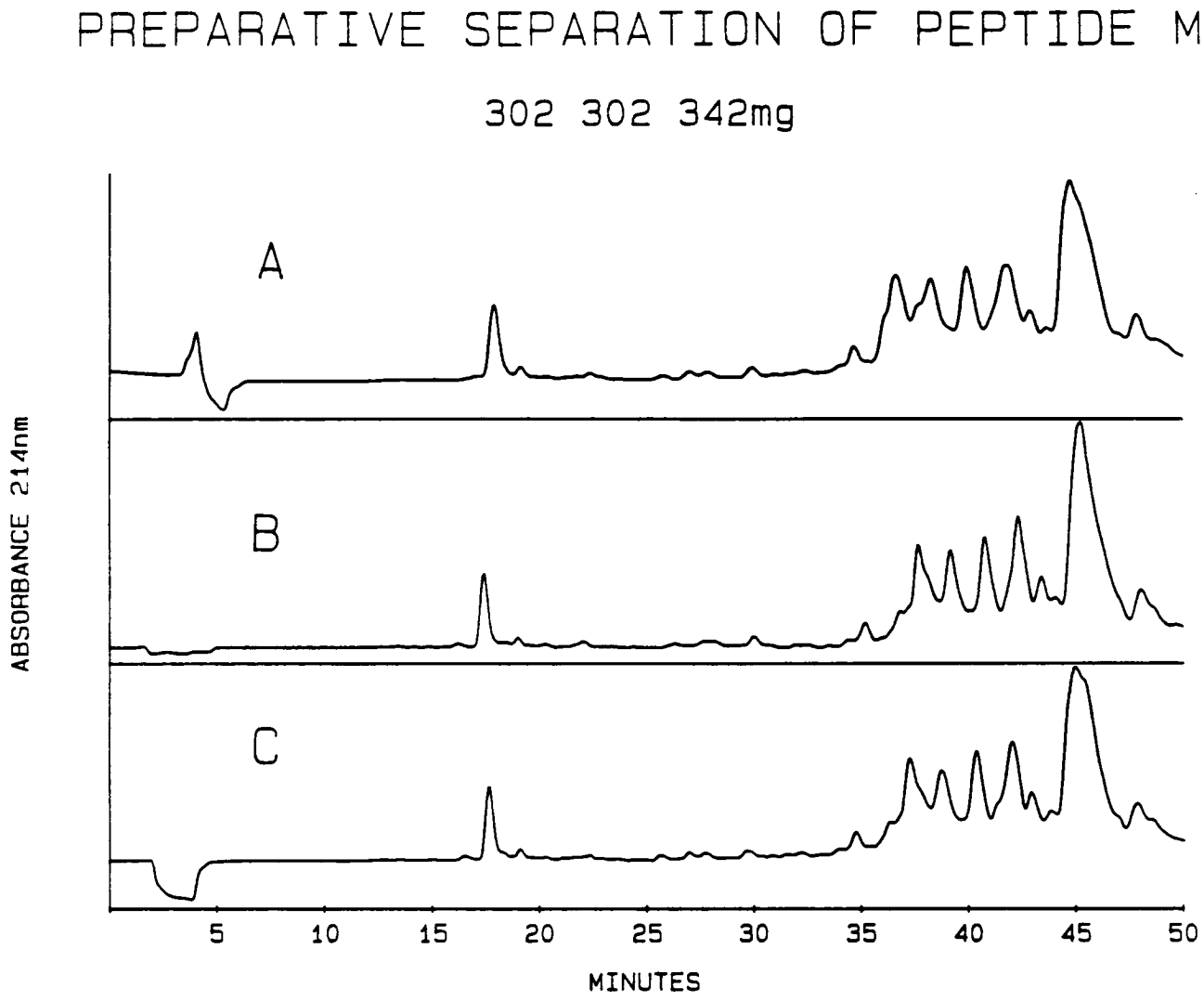


FIGURE 4. Preparative separations of synthetic Peptide M on a 50 mm x 30 cm Delta Pak, 300 A, 15 u, C18 column. Buffer A was 0.1% aqueous TFA and Buffer B was acetonitrile with 0.1% TFA. A 40 minute linear gradient from 0-25% B was run at a flow rate of 80 ml/min. The crude sample was dissolved in distilled water at a concentration of 2.5 mg/ml and filtered through a 0.45 uM Durapore membrane. The sample was applied to the column directly through the solvent delivery system and the column was re-equilibrated in Buffer A before the gradient was initiated. Detection was at 214nm at a sensitivity of 0.4 AUFS. PANELS A and B show the separations of 302 mg and PANEL C 342 mg.

RECHROMATOGRAPHY OF PREP FRACTIONS

COLLECTED AT 44.5 45 AND 47 MINUTES

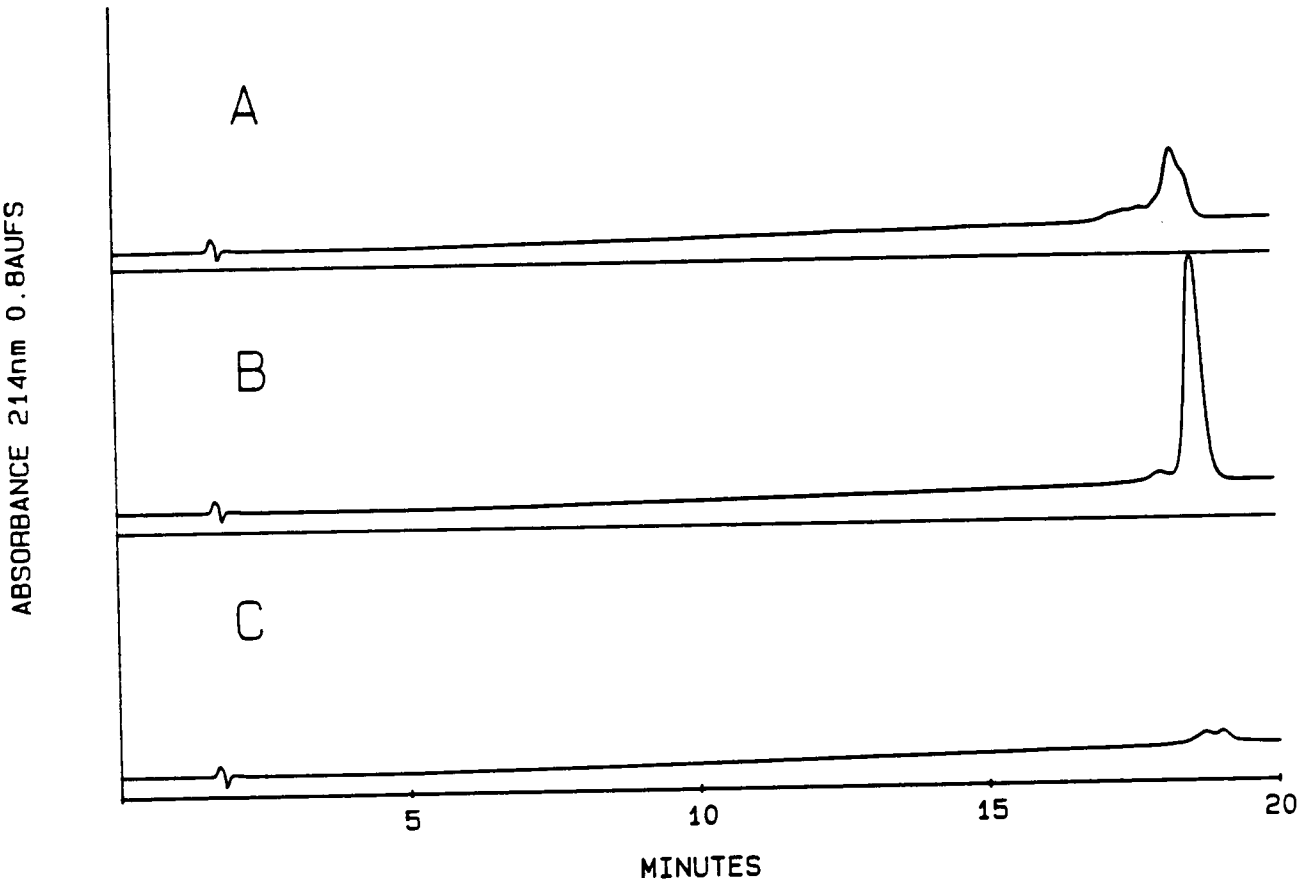


FIGURE 5. Rechromatography of fractions from the preparative separation (Figure 5, Panel C) of 342 mg of synthetic Peptide M on a 3.9 mm x 15 cm uBondapak C18 column. Buffer A was 0.1% aqueous TFA and Buffer B was acetonitrile with 0.1% TFA. A 15 minute linear gradient from 0-25% B was run at a flow rate of 1 ml/min. Fifty microliter aliquots of the preparative fractions were injected and detected at 214nm, 0.8 AUFS. PANEL A shows the fraction collected at 44.5 min, PANEL B 45 min and PANEL C 47 min. Peptide M eluted in fractions 45 (peak tube) through 46.5.