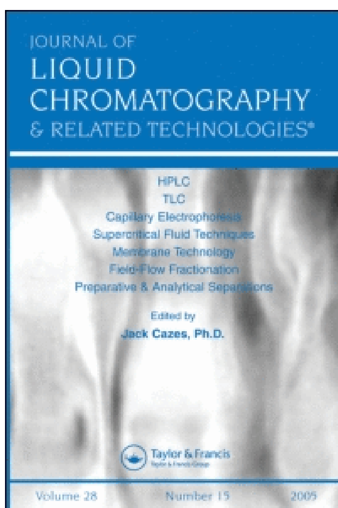
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Triethylamine Formate Buffer for HPLC-field Desorption Mass Spectrometry of Oligopeptides

D. M. Desiderio[a]; M. D. Cunningham[a]
[a] Department of Neurology and Charles B. Stout Neuroscience Mass Spectrometry Laboratory, University of Tennessee Center for the Health Sciences, Memphis, Tennessee

To cite this Article Desiderio, D. M. and Cunningham, M. D.(1981) 'Triethylamine Formate Buffer for HPLC-field Desorption Mass Spectrometry of Oligopeptides', Journal of Liquid Chromatography & Related Technologies, 4: 4, 721 — 733

To link to this Article: DOI: 10.1080/01483918108059968

URL: http://dx.doi.org/10.1080/01483918108059968

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(4), 721-733 (1981)

TRIETHYLAMINE FORMATE BUFFER FOR HPLC-FIELD DESORPTION MASS SPECTROMETRY OF OLIGOPEPTIDES

D.M. Desiderio and M.D. Cunningham

Department of Neurology and Charles B. Stout Neuroscience
Mass Spectrometry Laboratory, University of Tennessee
Center for the Health Sciences, 800 Madison Avenue,
Memphis, Tennessee 38163

ABSTRACT

A triethylamine-formate buffer system for the HPLC analysis of mixtures of oligopeptides is described. The volatility of TEAF facilitates buffer removal for subsequent field desorption-mass spectral qualitative and quantitative analyses. TEAF permits femtomole quantification of somatostatin, is UV transparent and enables high resolution separation of oligopeptide mixtures.

Downloaded At: 18:43 24 January 2011

INTRODUCTION

Reverse phase (RP) high performance (pressure) liquid chromatography (HPLC) is assuming an increasingly pivotal role in resolution of mixtures of biologic oligopeptides (1-20). Quantification of underivatized oligopeptides by field desorption mass-spectral (FD-MS) techniques at the pmol level signals a need for volatile HPLC buffers (21). Requirements for a volatile buffer in our research program include ultraviolet transparency down to 190 nm, high resolution (22) and sensitivity to less than one ng peptide. This paper describes for the first time development of a triethylamine-formic acid (TEAF) buffer system for RP-HPLC which meets these requirements.

Copyright © 1981 by Marcel Dekker, Inc.

Formic acid-pyridine buffers were utilized in separation of opioid peptide mixtures (23,24), while formic acid-methanol was used for endorphin purification (25). Trifluorocetic and formic acids were compared in separation of opioids and opioid peptides (26). Rivier mentions use of TEAF both in isolation of somatostatin from pigeon pancreas (27) and while describing trialkylammonium phosphate buffers in HPLC (9), but problems due to lack of sensitivity in the 200-230 nm region were noted. However, high concentrations (0.25M) TEAF were utilized in those studies. Data from our laboratory using a triethylamine-phosphate (TEAP) buffer system indicated excellent resolution of oligopeptides, down to five ng SS, and speed of separation (22). However, this buffer system is not volatile and interferes with subsequent FD-MS analysis.

Downloaded At: 18:43 24 January 2011

Ammonium acetate-acetonitrile was used to separate enkephalins, endorphins and analogs on a μ-alkylphenyl column (28). Trifluorocetic acid (0.1%) optimized separation of peptides containing two to 32 amino acids (29). An optimum RP-HPLC system was evaluated and 10-20 ng somatostatin (SS) was determined using 210 nm as detection wavelength (30). A detailed explanation of UV detection at 191-194 nm underlines the importance of removing chloride which also absorbs strongly in that region (31). A recent publication outlines a proposed retention mechanism for RP-HPLC wherein it is established by conductometric measurement that ion-pair formation does not occur (32).

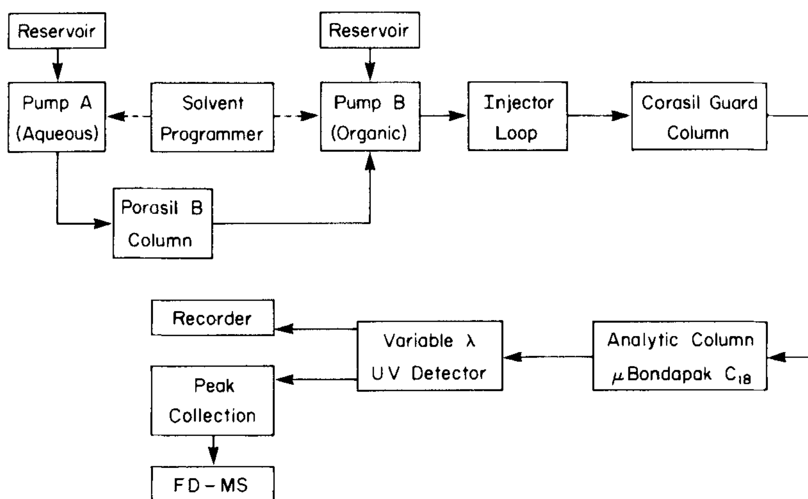
This paper describes 0.04M TEAF as an appropriate buffer for eventual use in RP-HPLC-FD-MS quantification of underivatized oligopeptides. This buffer system is volatile, UV transparent,

resolves peptides within minutes and permits determination of peptides down to the 600 femtomole (fmol) level.

MATERIALS AND METHODS

A Waters (Milford, MA) HPLC system was employed (see schematic). This system was outfitted with a U6K injector, 660 solvent programmer, two 6000A pumps and a 450 variable wavelength UV detector. A guard column packed with 37-50μ Bondapak C_{18}-Corasil was inserted in-line after the solvent pumps and before the analytic column to protect the latter when injecting biologic extracts. A 30 cm column packed with 37-75μ Porasil B was placed between the solvent pumps to absorb any TEAF impurities that might be present.

Downloaded At: 18:43 24 January 2011



SCHEMATIC

Schematic representing sample and buffer flow through chromatograph illustrating pumps, programmers, aqueous clean-up column, injector, guard and analytic columns, UV detector, peak collection and recorder.

A Laboratory Data Control (Riviera Beach, FL) Spectromonitor III variable wavelength UV detector was employed during high sensitivity measurements of this study. This detector has higher sensitivity specifications (0.005 AUFS) compared to the Waters 450 detector (0.01 AUFS).

A Waters μBondapak C_{18} column (registry no. 105142) was employed and a Precision Sampling (Baton Rouge, LA) Pressure-Lok series B-110 25 μl syringe used for sample injection. The column was washed each night with either CH_3OH or CH_3CN. Unsilanized glassware was employed.

Downloaded At: 18:43 24 January 2011

Acetonitrile (Lot AE397) was purchased from Burdick and Jackson (Muskegon, MI), formic acid (Lot FL12A979) from Matheson, Coleman & Bell (Cincinnati, OH) and triethylamine (Lot 59C-0352) from Sigma Chemical Company (St. Louis, MO). Triethylamine (150 ml) was redistilled before use and the fraction distilling at 87.5^{o}C was collected and stored under nitrogen in screw-top vials before use. Laboratory deionized water was employed.

Somatostatin (SS) was purchased from Bachem (Torrance, CA), while bradykinin, luteinizing releasing hormone (LRH), neurotensin, met-enkephalin, angiotensin II, leu-enkephalin, substance P and eledoisin-related peptide were purchased from Sigma. Buffer was prepared by titrating 0.04M formic acid with triethylamine to pH 3.15. Other experimental conditions where otherwise noted: 200 nm; -5 offset; flow rate = 1.5 ml/min; pressure 1200-1300 p.s.i. at 25-26% CH_3CN and 1100 p.s.i. at 34-35%.

Mobile phases were aspirated through 0.47μ cellulose acetate filters (HAWP04700, Millipore, Bedford, MA) and organic solutions through 0.5μ fluorocarbon filters (FHUP04700). Chart speed 0.2 cm/min.

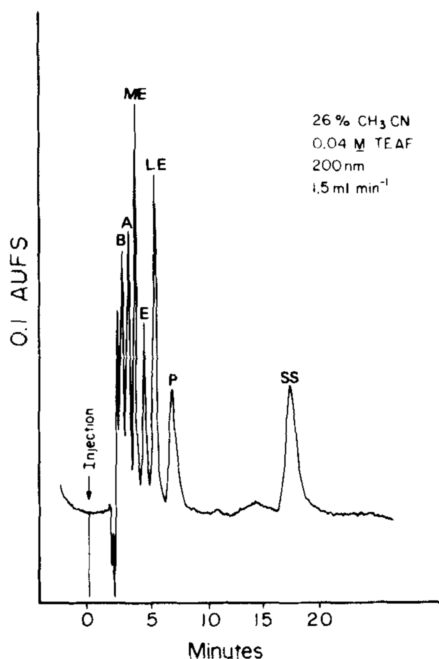
Factory-packed small disposable cartridges (Sep-Pak$^{(R)}$) filled with Bondapak C_{18} (70μ) were purchased from Waters. Time equivalent to the void volume (t_o) was determined by measuring the time between injection and the first baseline disturbance or by using the formula t_o=LD/1.57 (33) where d is the column i.d. (0.39 cm), F is solvent flow rate (1.5 ml min^{-1}) and L = 30 cm. A value of t_o = 2.01 is calculated and corresponds to the measured t_o = 1.6 min.

Analog UV detector output was recorded on a Houston Instrument Omniscribe Recorder Model B5217-1 (Houston, TX) dual-pen strip-chart recorder 10 mV FS.

Downloaded At: 18:43 24 January 2011

RESULTS AND DISCUSSION

Figure 1 contains an HPLC chromatogram illustrating isocratic 26% CH_3CN resolution of a mixture of seven oligopeptides - bradykinin, angiotensin II, leu-enkephalin, eledoisin-related peptide, met-enkephalin, substance P and somatostatin. Good resolution and speed of separation are observed in this chromatogram. Additional peptides are easily and completely resolved whenever necessary by either simply adjusting the isocratic elution conditions or by running a solvent gradient.



Downloaded At: 18:43 24 January 2011

FIGURE 1

HPLC separation of mixture of seven oligopeptides with TEAF buffer: 500 ng each of bradykinin (B), angiotensin II (A), methionine-enkephalin (ME), eledoisin-related peptide (E) and leucine-enkephalin (LE); 1 µg each of substance P (P) and somatostatin (SS). 26% CH_3CN: 74% TEAF; 200 nm; 0.1 AUFS; 1.5 ml min^{-1}; 1200 psi.

Linear variation of the logarithm of the capacity factor (k') for several peptides versus percentage of organic modifier is shown in Figure 2. Extensive data are shown for LRH and bradykinin, while limited data are given for the other oligopeptides. Visual inspection shows all slopes are generally equal.

Data in Figure 3 represent the linear relationship between peak height of an HPLC peak and amount of somatos-

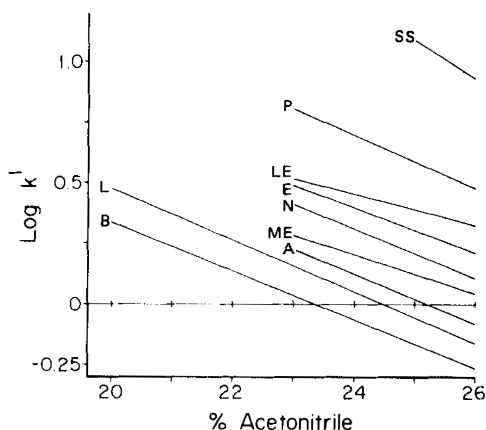


FIGURE 2

Linear variation of logarithm of k' versus percentage of organic modifier.

Downloaded At: 18:43 24 January 2011

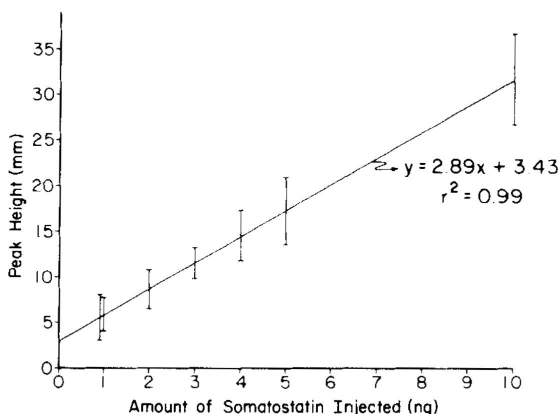


FIGURE 3

Linear relationship observed between recorded peak height (mm) and amount (ng) of injected somatostatin (34% CH_3CN). Linear regression and correlation coefficient are shown. Other experimental parameters are given in the text. Vertical lines indicate standard deviations. Number of determinations are: 7, 10, 7, 9, 7, 5 and 4 for 10 ng, 5, 4, 3, 2, 1 and 0.9, respectively.

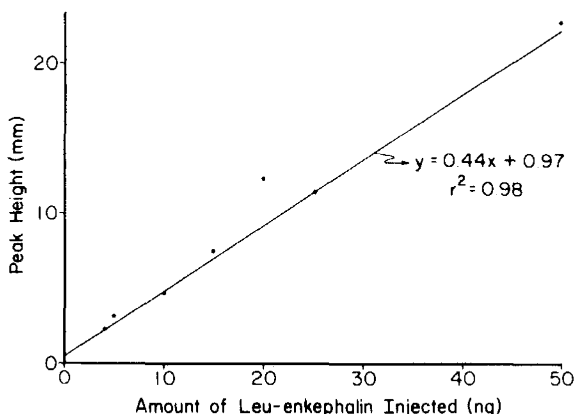


FIGURE 4

Linear relationship observed between recorded peak height (mm) and amount (ng) of leu-enkephalin injected (26% CH_3CN). Linear regression and correlation coefficient are shown. Other experimental parameters are given in the text.

Downloaded At: 18:43 24 January 2011

tatin injected. Linearity is observed from below one ng to ten ng. The correlation coefficient for this regression line is 0.99. Vertical lines represent $\pm$ standard deviation obtained from multiple injections (see legend). The lowest amount of SS injected producing a peak having the appropriate retention time and approximately a 1:1 signal-to-noise ratio is represented by 0.9 ng or 552 fmol. By comparison, the amount of SS in one rat hypothalamus is 40 ng (34).

The linear relationship between HPLC peak height and amount of injected leu-enkephalin is given in Figure 4 and for met-enkephalin in Figure 5. Linearity is observed in both cases from one to 50 ng.

Data in Figure 6 represent original recordings of sequentially lower amounts of somatostatin injected. Good peak shapes are

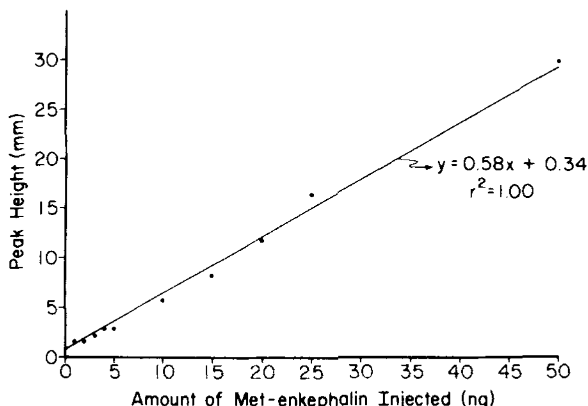


FIGURE 5

Linear relationship observed between recorded peak height (mm) and amount (ng) of met-enkephalin (26% CH_3CN). Linear regression and correlation coefficient are shown. Other experimental parameters are given in the text.

Downloaded At: 18:43 24 January 2011

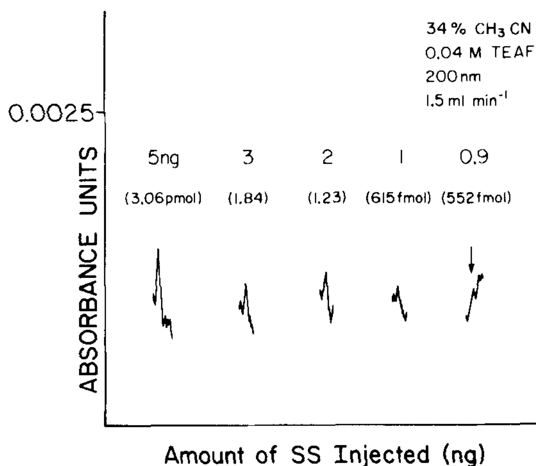


FIGURE 6

Original traces of 5, 3, 2, 1 and 0.9 ng injected SS. 34% CH_3CN: 66% 0.04M TEAF; 200 nm; 0.005 AUFS; 1.5 ml min^{-1}; 1100 psi. Arrow on 0.9 ng injection denotes known SS retention time.

observed. These data illustrate femtomole sensitivity obtainable with a 0.04M TEAF buffer system. In the more dilute solutions used in this study vis-a-vis more concentrated solutions (0.25M) of other studies, it can be seen UV transparency, sensitivity and resolution properties of the current TEAF buffer are excellent.

CONCLUSIONS

A volatile TEAF buffer system capable of high resolution and femtomole sensitivity is described. Regression lines were obtained enabling quantification of endogenous levels of biologic oligopeptides. The ability to remove TEAF buffer by lyophilization permits analysis of underivatized oligopeptides by FD-MS where three pmol of leu-enkephalin have been quantified recently (21).

Work is in progress in our laboratory with extraction, purification and quantification of biologic oligopeptides in hypothalamic, dental and brain tissue. After protein precipitation with perchloric acid, samples are prepurified with RP Sep-Paks (R) (35-38). Samples may be directed to either FD-MS quantification or, on the other hand, to further chromatographic purification and/or quantification with HPLC. HPLC eluates can also be collected and subjected to FD-MS quantification. This research program is being undertaken to provide an independent assay method to verify radioimmunoassay (RIA) results (39). FD-MS quantitative data are based upon intact molecular structure of the peptide while RIA may be sensitive only to a portion of the

Downloaded At: 18:43 24 January 2011

peptide. Development of the described TEAF buffer system is an obligatory component in this overall research scheme. TEAF buffer has been shown to possess sufficient resolving power, speed of analysis, sensitivity, volatility and UV transparency. Equipment and columns are long-lasting and no degradation of resolution is observed even after injection of several biologic samples. Fmol sensitivity is obtainable and no derivatization is required as UV detection at 190-210 nm is universal for peptide bonds.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge generous financial support from USPHS (NIH GM 26666).

Downloaded At: 18:43 24 January 2011

REFERENCES

1. W.S. Hancock, C.A. Bishop, L.J. Meyer and D.R.K. Harding, J. Chrom., 161, 291 (1978).

2. W.S. Hancock, C.A. Bishop, R.L. Prestidge, D.R.K. Harding and M.T.W. Hearn, J. Chrom., 153, 391 (1978).

3. W.S. Hancock, C.A. Bishop, R.L. Prestidge and M.T.W. Hearn, Anal. Biochem., 89, 203 (1978).

4. W.S. Hancock, C.A. Bishop and M.T.W. Hearn, FEBS Letters, 72, 139 (1976).

5. M.T.W. Hearn, W.S. Hancock, J.G.R. Hurrell, R.J. Fleming and B. Kemp, J. Liq. Chrom., 2, 919 (1979).

6. J.A. Feldman, M.L. Cohn and D. Blair, J. Liq. Chrom., 1, 833 (1978).

7. E. Lundanes and T. Greibrokk, J. Chrom., 149, 241 (1978).

8. J. Rivier, R. Wolbers and R. Burgus, Proc. Fifth Amer. Pept. Sympos., 52 (1977).

9. J.E. Rivier, J. Liq. Chrom., 1, 343 (1978).

10. H.R. Morris, A.T. Etienne, A. Dell and R. Albuquerque, J. Neurochem., 34, 574 (1980).

11. D.D. Blevins, M.F. Burke and V.J. Hruby, Anal. Chem., 52, 420 (1980).

12. B. Larsen, V. Viswanatha, S.Y. Chang and V.J. Hruby, J. Chrom. Sci., 16, 207 (1978).

13. W.A. Schroder, J.B. Shelton, J.R. Shelton and D. Powars, J. Chrom., 174, 385 (1979).

14. T.A. Stoming, F.A. Garver, M.A. Gangarosa, J.M. Harrison and T.H.J. Huisman, Anal. Biochem., 96, 113 (1979).

15. R.A. Martinelli and H.A. Scherage, Anal. Biochem., 96, 246 (1979).

16. H.P.J. Bennett, C.A. Browne, P.L. Brubaker and S. Solomon, Proc. Third Intl. Liq. Chromatog. Symp., 1979 (in press).

17. W. Monch and W. Dehnen, J. Chrom., 147, 415 (1978).

18. I. Molnar and C. Horvath, J. Chrom., 142, 623 (1977).

19. M.J. O'Hare and E.C. Nice, J. Chrom., 171, 209 (1979).

20. M.E.F. Biemond, W.A. Sipman and . Olivie, J. Liq. Chrom., 2, 1407 (1979).

21. D.M. Desiderio, S. Yamada, F. Tanzer and J.Z. Sabbatini, Biomed. Mass Spectrom., (submitted).

22. D.M. Desiderio, J.L. Stein, M.D. Cunningham and J.Z. Sabbatini, J. Chrom., (in press).

23. R.V. Lewis, S. Stein and S. Udenfriend, Int. J. Pept. Prot. Res., 13, 493 (1979).

24. A.S. Stern, R.V. Lewis, S. Kimura, J. Rossier, L.D. Gerber, L. Brink, S. Stein and S. Udenfriend, Proc. Natl. Acad. Sci., 76, 6680 (1979).

25. S. Gentleman, L.I. Lowney, B.M. Cox and A. Goldstein, J. Chrom., 153, 274 (1978).

26. C.E. Dunlap, S. Gentleman and L.I. Lowney, J. Chrom., 160, 191 (1978).

27. J. Spiess, J.E. Rivier, J.A. Rodkey, C.D. Bennett and W. Vale, Proc. Natl. Acad. Sci., 76, 2974 (1979).

Downloaded At: 18:43 24 January 2011

28. B.L. Currie, J.K. Chang and B. Cooley, J. Liq. Chrom., 3, 513 (1980).

29. D. Voscamp, C. Olieman and H.C. Beyerman, Recl. Trav. Chim. Pays-Bays 99, 105 (1980).

30. M. Abrammson and K. Groningsson, J. Liq. Chrom., 3, 495 (1980).

31. M.M. Mayer and J.A. Miller, Anal. Biochem., 36, 91 (1970).

32. B.A. Bidlingmeyer, S.N. Deming, W.P. Price, B. Sachok and M. Petrusek, J. Chromatog. 186, 419 (1979).

33. Chromatography, D. Heftman (Ed), Van Nostrand-Reinhold, New York (1975).

34. L.L. Iversen, R.A. Nicoll and W.W. Vale, Neurosci. Res. Prog. Bull. 16, 211 (1978).

35. H.P.J. Bennett, C.A. Browne, P.L. Brubaker and S. Solomon, Proc. Third Int. Liq. Chrom. Symp. (G. Hawk, Ed), in press.

36. R.A. Hartwick, D. Van Haverbeke, M. McKeag and P.R. Brown, J. Liq. Chromatog. 2, 725 (1979).

37. J.O. Whitney and M.M. Thaler, J. Liq. Chromatog., 3, 545 (1980).

38. M.J. Fasco, M.J. Cashin and L.S. Kaminsky, J. Liq. Chromatog., 2, 565 (1979).

39. See, for example, W.W. Youngblood, M.A. Lipton and J. S. Kizer, Brain. Res., 151, 99 (1978).

Downloaded At: 18:43 24 January 2011