Department of Comparative Medicine[1] and Department of Neuroscience and Anatomy[2], M. S. Hershey Medical Center, Hershey, PA, U. S. A., and Bioanalytical and Drug Development Laboratory[3], Biological and Medical Research Department King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

A rapid sample preparation method for the HPLC determination of the opioid antagonist naltrexone in serum

W. J. Hurst[1], I. S. Zagon[2] and H. Y. Aboul-Enein[3]

HPLC with UV and electrochemical detection has routinely been employed for the determination of the opioid antagonist naltrexone in serum. Sample preparation protocols range from liquid/liquid to solid phase extraction. The sample preparation described in this communication uses ultrafiltration as the mode of sample preparation prior to HPLC analysis. The method is accurate, precise and saves considerable time compared to previously published techniques.

1. Introduction

Naltrexone hydrochloride is a well known opioid antagonist that is used in a variety of clinical and research applications [1]. This opioid antagonist has previously been analyzed by a variety of methods including chemiluminescence [2], TLC, GC-NPD, GC-MS and HPLC [3–16] with HPLC being the method of choice. The HPLC methods that have been developed have used UV, MS or electrochemical detectors [5, 6, 9, 12, 13, 16–18].

Sample preparation for the determination of naltrexone and related compounds in blood and other biological fluids have been varied with liquid/liquid and solid phase extraction methods being used. In the case of the liquid/liquid extraction, samples of alkalized blood were initially extracted with ethyl ether with the compounds of interest then being extracted into phosphoric acid before HPLC analysis. The liquid/liquid extraction methods have largely been replaced by solid phase extraction (SPE) protocols on ODS supports where samples are absorbed onto the columns, washed with some intermediate support and the naltrexone containing fraction eluted with 100% methanol [14–16].

In the present study, we report an alternative method where samples are ultrafiltered through a 10,000 MW membrane prior to HPLC analysis with UV detection. The method is straightforward and provides essentially 100% recovery from a biological matrix.

2. Investigations, results and discussion

Samples of the naltrexone standard were analyzed before and after passing through the UF membrane. After multiple samples, n = 7, 99.4% recovery was obtained. Samples of naltrexone in bovine serum albumin (BSA) also were

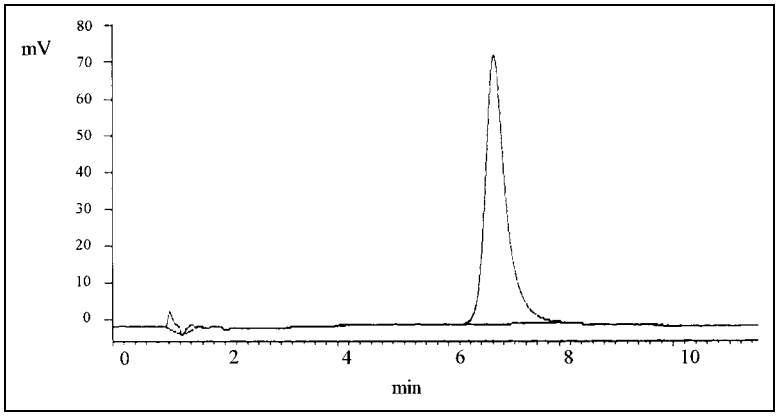

Fig. 1:
Naltrexone standard (50 ng) chromatogram

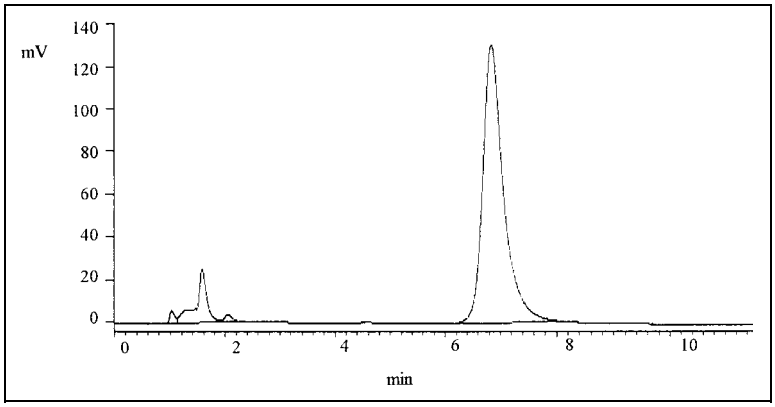

Fig. 2:
Chromatogram of biological matrix spiked with naltrexone

passed through the UF membrane with recoveries of 95.5% observed (n = 7). Multiple injections of a standard and sample (n = 8), were made to assess the method precision with relative standard deviations (RSDs) of 3.5 and 4.2% respectively. Lower limits were calculated at 2 times signal to noise ratio (s/n) and found to be 2 ng, which is equivalent to data reported for HPLC methods with electrochemical detection.

The reported method is simple, economical, straightforward, less time consuming and can also be applied to small volumes of biological fluids. It can be applied to other biological fluids for sample preparation prior to naltrexone analysis.

3. Experimental

3.1. Apparatus

The HPLC used consisted of a Waters 510 solvent delivery system at a flow rate of 0.75 ml/min and a Waters Model 480 UV detector monitoring 210 nm. A Waters Model 710 WISP was used for sample injection (Waters; Milford, MA). The column used for these determinations was an ODS-5-80, C-18 (4.6×150 mm) from Separation Methods Technologies (Wilmington, DE).

3.2. Reagents

All reagents used were HPLC grade. The mobile phase consisted of a 50 : 50 : 0.1 (v/v/v) mixture of methanol/water/85% phosphoric acid containing 0.03 mM octanesulfonic acid and was similar to that used by Wilson [18]. Bovine serum albumin (BSA), type IV, was obtained from Sigma (St. Louis, MO, USA) and used to prepare a simulated biological matrix.

3.3. Naltrexone standard

The naltrexone (Sigma, St. Louis, MO, USA) standard was prepared in mobile phase at a concentration of 5 ng/µl and stored frozen in 5 ml aliquots. At the time of use, this preparation was thawed, thoroughly mixed and stored in a refrigerator. This procedure was evaluated over a several month period to ensure that no spurious peaks would result.

3.4. Simulated samples

Naltrexone was added a 2 µg/ml to a 2% solution of BSA in phosphate buffer solution (pH 7.4) to evaluate the potential matrix effects. This sample was mixed thoroughly to ensure sample homogeneity.

3.5. Sample preparation

Samples were placed into Millipore UF units with 10,000 MW cutoff cellulose membrane and used according to the manufacturers guidelines [19]. The first three drops were allowed to waste and the remaining effluent collected in the accompanying collection vessel. After approx. 15 min, samples were withdrawn from the collection vessel for subsequent HPLC analysis.

3.6. HPLC analysis

Naltrexone (50 ng) was injected and had a retention time of approximately 6.7 min. Fig. 1 provides an example chromatogram of the standard material. After sample preparation, 50 µl amounts of the sample were injected (Fig. 2) which were equivalent to 100 ng drug.

Acknowledgements. Supported by NIH Grant EY10300 with special thanks to Dr. Max Lang, Chairman of the Department of Comparative Medicine for his encouragement and support.

References

1 Physician's Drug Handbook, 5th Edition, p. 740, Springhouse Corp., Springhouse, PA 1995
2 Campiglio, A.: Analyst **123**, 1053 (1998)
3 Meffin, P. J.; Smith, K. J.: J. Chromatogr. **183**, 352 (1980)
4 Verebely, K.; Alarazi, J.; Lehrer, M.; Mule, S. J.: J. Chromatogr. **378**, 261 (1986)
5 O'Conner, E. F.; Cheng, S. W.; North, W. G.: J. Chromatogr. **491**, 240 (1989)
6 Chou, J. Z.; Albeck, H.; Kreek, M. J.: J. Chromatogr. **613**, 359 (1993)
7 Asali, L. A.; Nation, R. L.; Brown, K. F.: J. Chromatogr. **278**, 329 (1983)
8 Sprague, G. L.; Takemori, A. E.: J. Pharm. Sci. **68**, 660 (1979)
9 Verebely, K.; Mule, S. J.; Jukofsky, D.: J. Chromatogr. **111**, 141 (1975)
10 Burce, G. L.; Bhat, H. B.; Sokoloski, T.: J. Chromatogr. **137**, 323 (1977)
11 Sams, R. A.; Malspeis, L.: J. Chromatogr. **125**, 409 (1976)
12 Kintz, P.; Mangin, P.; Lugnier, A. A.; Chaumont, A. J.: Z. Rechtsmed. – J. Legal Med. **103**, 57 (1989)
13 Peh, K. K.; Billa, N.; Yuen, K. H.: J. Chromatogr. B **701**, 140 (1997)
14 Pacifici, R.; Pichini, S.; Altieri, I.; Caronna, A.; Passa, A. R.; Zuccaro, P.: J. Chromatogr. B **664**, 329 (1995)
15 Zuccaro, P.; Altieri, I.; Betto, P.; Pacifici, R.; Ricciarello, G.; Pini, L.; Sternieri, E.; Pichini, S.: J. Chromatogr. **567**, 485 (1991)
16 Reid, R. W.; Deakin, A.; Leehey, D. J.: J. Chromatogr. **614**, 117 (1993)
17 Grauer, S. M.; Tao, R.; Auerbach, S. B.: Brain Res. **599**, 277 (1992)
18 Wilson, T. D.: J. Chromatogr. **298**, 131 (1984)
19 Instructions for Millipore LGC Filter Units; Millipore Corp., Bedford, MA

Received October 22, 1998
Accepted December 18, 1998

Hassan Y. Aboul-Enein, Ph. D.
Biological and Medical Research Dept. (MBC-03)
King Faisal Specialist Hospital and Research Centre
P. O. Box 3354
Riyadh 11211
Saudi Arabia
enein@kfshrc.edu.sa