Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 1055–1058, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
© 1990 Pergamon Press plc

Short Communication

The analysis of atenolol in plasma using an automated sample preparation technique*

A.D. DALE† and S.E. TURNER

Biokinetix Ltd, Potters Bar, Hertfordshire, UK

Keywords: *Atenolol; liquid chromatography; plasma; automated sample preparation.*

Introduction

The adrenoreceptor blocking drugs are of therapeutic use in the treatment of several cardiac conditions including angina pectoris and cardiac arrythmia [1, 2]. Most of this group of drugs are aryloxypropanolamines, of which atenolol is a highly lipophilic example. Atenolol, [4-(2-hydroxy-3-isopropylaminopropoxy)-phenyl-acetamide], was introduced in the 1970s for the treatment of hypertension. Subsequent experiments in both animals and humans indicated that it is free of any significant membrane-sensitizing activity and has no intrinsic sympathomimetic activity [3]. Atenolol has been determined in plasma and urine by gas–liquid chromatography [4] and, more recently, by reversed-phase HPLC, with either UV [5] or fluorescence detection [6]. The majority of these HPLC assays have relied on the initial denaturation of the plasma proteins by the addition of sodium hydroxide solution [7], followed by extraction of the drug into an immiscible organic solvent. Several solvents, or mixtures of solvents, have been described for this purpose, including ethyl acetate [6], methyl *t*-butyl ether [7], dichloromethane (Dale and Turner, unpublished results) and a mixture of cyclohexane with 1-butanol [8].

Such methods are labour intensive and require about 1.5 h for the preparation of samples, although, in practice, several samples can be processed simultaneously. In addition, relatively expensive apparatus is required with these potentially hazardous solvents, especially when the solvent is being evaporated to dryness. More recently, the use of a solid-phase extraction technique has been described for the estimation of atenolol in plasma, but the method does not lend itself easily to automation, which is almost essential when large numbers of samples are being handled.

This paper describes the use of an ASTED (automated sequential, trace enrichment dialysate) sample processor to isolate atenolol, at therapeutic levels, from human plasma. The assay is fully automated and the extraction proceeds concurrently with the chromatographic analysis, thus reducing the overall analysis time. This novel method is compared with a solvent extraction method.

Principles of the ASTED

The ASTED performs the following operations automatically: (1) the sample, with the internal standard if necessary, is transferred to one side of a semi-permeable membrane with a molecular weight cut-off of about 15,000; (2) on the other side of the membrane is a recipient solution, the composition of which is varied according to the application, and into which the drug and the internal standard diffuse; (3) after allowing for this diffusion, the recipient solution is pumped through a short column packed with silica bonded with octadecyl moieties, which retains the compounds of interest; (4) this pre-column is then placed in line with the analytical column, using conven-

* Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.
† Author to whom correspondence should be addressed.

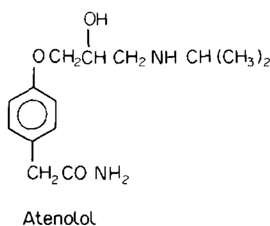
tional column switching techniques and the various retained compounds are desorbed from the short column and transferred as a tight band to the top of the analytical column.

Experimental

Materials and methods

Atenolol hydrochloride was supplied by Generics UK Ltd, (Potters Bar, Herts, UK) and the procainamide hydrochloride, used as the internal standard, from Sigma Chemical Co. Ltd (Poole, Dorset, UK). The structures of these compounds are shown in Fig. 1.

Other reagents used were of analytical grade and supplied by FSA (Loughborough, UK). The water was deionized by an Elga reversed-osmosis water purification system (Elga, High Wycombe, Bucks, UK). The ASTED was supplied by Anachem (Luton, Bedfordshire, UK) and was fitted with a 15K-Dalton semi-permeable membrane. The trace enrichment cartridge was packed with octadecyl-bonded silica. The chromatograph was modular, comprising a Spectra-Physics solvent pump (Spectra-Physics, Hemel Hempsted, Herts, UK), a WISP autosampler (Waters Associates, Watford, Herts, UK) and a Perkin Elmer fluorescence detector Model LS40 (λ_{ex}: 280 nm, λ_{em}: 320 nm) (Perkin Elmer, Beaconsfield, Bucks, UK). The output of the detector was fed to a Spectra-Physics Model 4200 recording integrator. The columns used were a LiChrosorb CN pre-column and an Apex II cartridge (15 × 0.4 cm), packed with cyano-bonded silica (Jones Chromatography, Hengoed, Mid-Glamorgan, Wales, UK).



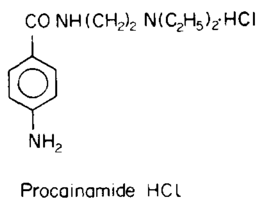


Figure 1
Chemical structures of atenolol and procainamide.

Solvent extraction

To the plasma (1 ml) was added the internal standard solution with mixing. The plasma proteins were then denatured by the addition of 10 M sodium hydroxide solution and the mixture extracted with dichloromethane by shaking and centrifuging.

The upper phase was discarded and the organic phase evaporated to dryness under nitrogen. The residue was dissolved in a small volume of mobile phase and injected onto the analytical column.

ASTED extraction

The conditions for the ASTED extraction were as follows: injection volume, 100 μl; sample diluent, 0.9% sodium chloride solution; recipient solution, 100 mM disodium hydrogen phosphate adjusted to pH 7.0 with phosphoric acid; enrichment volume, 6000 μl.

Chromatographic conditions

The mobile phase conditions for the two extraction procedures differed slightly in the composition of the eluent. For the solvent extraction method the mobile phase was methanol–water–acetic acid–triethanolamine–octane sulphonic acid, sodium salt (20:80:0.3:0.1:0.05, v/v/v/v/w).

The eluent for ASTED extraction was methanol–water–acetic acid–triethanolamine–octane sulphuric acid, sodium salt (40:60:0.3:0.1:0.05 v/v/v/v/w).

Results and Discussion

As may be seen from Figs 2 and 3, both extraction methods give acceptable results. The composition of the eluent had to be changed slightly to resolve an interfering peak, but a linear detection response for the peak-height ratio (P) against concentration (C) was found for both methods over the range 25–1000 ng ml^{-1} in plasma, according to equations (1) and (2).

Solvent extraction method:

$$P = 0.00259C - 0.00183;\ r = 0.999;\ n = 6. \quad (1)$$

ASTED method:

$$P = 0.00104C - 0.00860;\ r = 0.998;\ n = 6. \quad (2)$$

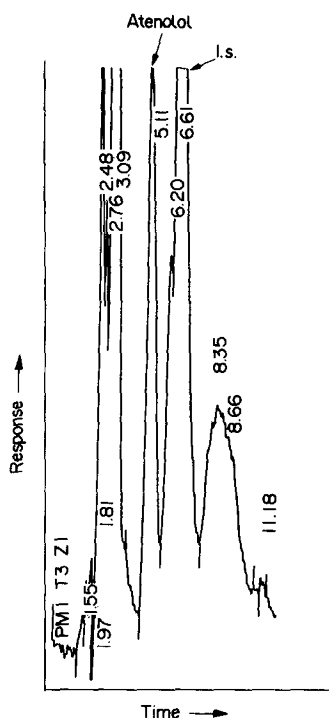


Figure 2
Chromatogram of 750 ng ml^{-1} atenolol in human plasma using an ASTED.

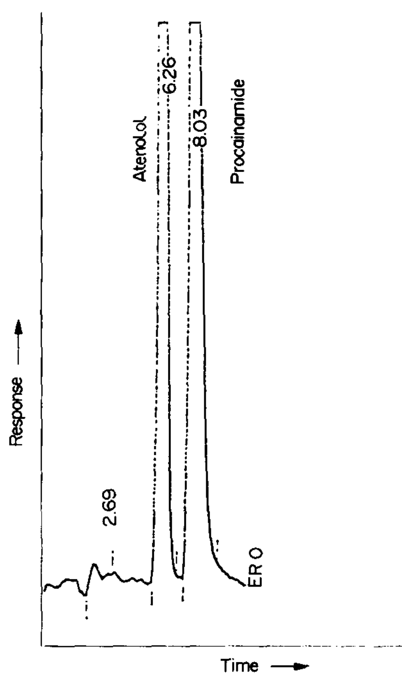


Figure 3
Chromatogram of 750 ng ml^{-1} atenolol in human plasma using solvent extraction.

The recoveries of the drug and internal standard for the two extraction procedures were estimated by comparing the peak heights obtained by direct injection of standard solutions with those obtained by the extraction of spiked plasma standards, allowances being made for the differing volumes.

For the solvent extraction method, the recoveries of atenolol and procainamide were 70.0 and 56.0%, respectively. For the ASTED method, the recoveries of atenolol and procainamide were 55.0 and 43.0%, respectively.

The relatively low recovery found for the ASTED did not significantly increase the lowest detectable limit of the compounds, which was about 5 ng ml^{-1} at a signal to noise ratio of 3:1.

The significant advantages of the ASTED method over the more conventional solvent extraction method lie in the relative ease and speed of extraction found in the automated technique. It has been found that it requires about 1 man-hour to prepare 100 samples of plasma for the ASTED method compared with about 5 man-hours required for the same number of samples prepared by the solvent extraction technique.

Another advantage of the ASTED approach is that it avoids the strongly alkaline conditions required for solvent extraction. This is especially important in the case of the β-blockers, which may be absorbed onto the glass vessels under conditions of high pH.

Conclusions

A novel method has been described for the estimation of the β-blocking drug, atenolol, in human plasma. This new extraction procedure has been compared with solvent extraction and it has been shown that the results from the new automated method are effectively identical with those obtained from the solvent extraction approach. The time required to prepare the samples is significantly less for the automated method.

References

[1] J. Eriksen, K. Osnik and J. Bedieten, *Acta Med. Scand.* **201**, 579–584 (1977).
[2] G.H. Regardh and G. Johnsson, *Clin. Pharmacokinet.* **5**, 557–569 (1980).
[3] G. Coppi, P. Mosconi and V. Springolo, *Curr. Ther. Res.* **42**, 411–418 (1987).
[4] W.H. Wan and K.R. Monson, *J. Pharm. Sci.* **67**, 1340–1342 (1978).

[5] S. Decourt and B. Flouvar, *J. Chromatogr.* **174**, 258–263 (1979).
[6] L.G. Miller and D.J. Greenblatt, *J. Chromatgr.* **381**, 201–204 (1986).
[7] Y.-G. Yee, P. Rubin and T.F. Blaschke, *J. Chromatogr.* **171**, 357–362 (1979).
[8] R.K. Bhamra, K.J. Thorley, J.A. Vale and D.W. Holt, *Ther. Drug Monit.* **5**, 313–318 (1983).

[*Received for review 5 April 1990; revised manuscript received 17 May 1990*]