Journal of Chromatography, 339 (1985) 424—428
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2494

Note

Determination of 4-amino-3-(*p*-chlorophenyl)butyric acid (baclofen) in plasma by high-performance liquid chromatography

P.M. HARRISON*, A.M. TONKIN and A.J. McLEAN

Department of Clinical Pharmacology, Alfred Hospital, Commercial Road, Prahran, 3181 Melbourne (Australia)

(First received August 7th, 1984; revised manuscript received November 27th, 1984)

Baclofen, a derivative of γ-aminobutyric acid (GABA), is used to relieve symptoms of spasticity in patients suffering from multiple sclerosis and other spinal lesions [1, 2]. Existing assay methods based on either gas—liquid chromatography [1, 2] or gas chromatography—mass spectrometry [3, 4] require chemical derivatization steps prior to assay. This paper describes a rapid, simple high-performance liquid chromatographic (HPLC) method for assaying baclofen in plasma without derivatization.

EXPERIMENTAL

Materials and reagents

Baclofen was supplied by Ciba-Geigy (Sydney, Australia). Acetonitrile (HPLC grade) was obtained from Waters Assoc. (Sydney, Australia) and all other reagents were of analytical grade. C_{18} Bond-Elut™ columns (1 ml capacity) and a Vac-Elut™ manifold (Analytichem, Harbor City, CA, U.S.A.) were purchased from FSE Scientific (Melbourne, Australia).

The HPLC system consisted of a Constametric III pump and a SpectroMonitor III variable-wavelength UV detector (LDC, Riviera Beach, FL, U.S.A.), a Model 7125 injection valve with a 50-μl loop (Rheodyne, Berkeley, CA, U.S.A.) and an Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.). The chromatography was carried out on a 25 cm × 0.3 cm I.D. stainless-steel glass-lined column packed with 5-μm Spherisorb ODS silica (SGE Scientific, Ringwood, Victoria, Australia).

0378-4347/85/$03.30

Bond-Elut extraction procedure

Baclofen was extracted from plasma using 1-ml Bond-Elut columns packed with ODS silica (C_{18} columns). The columns were placed in luer fittings in the top of the Vac-Elut chamber which has the capacity for ten columns. A vacuum of 25—50 cmHg was applied to the manifold to effect the various stages of the extraction. Prior to use the columns were activated by washing with 2 × 1 ml acetonitrile followed by 2 × 1 ml of 0.1% orthophosphoric acid solution.

To extract baclofen from plasma, 1 ml of plasma (sample or standard) was passed through the activated C_{18} Bond-Elut column which was then washed with 0.1% solution of orthophosphoric acid (0.5 ml) followed by 0.5 ml of 0.1% orthophosphoric acid—acetonitrile (80:20). The vacuum was then released and the stainless-steel needles of the Vac-Elut chamber wiped. Appropriately labelled tubes were placed under the columns, which were then eluted with 0.3 ml of 0.05 *M* sodium dihydrogen orthophosphate—acetonitrile (75:25). An aliquot (50 µl) of these extracts was then injected onto the HPLC column.

Chromatography

In order to separate baclofen from other endogenous components of plasma the HPLC column was eluted with a mobile phase of 0.05 *M* sodium dihydrogen orthophosphate—acetonitrile (95:5) at a flow-rate of 1 ml/min. Baclofen was detected at a wavelength of 220 nm and a sensitivity of 0.01 a.u.f.s.

Preparation of standards

Stock solutions of baclofen (1 mg/ml) were prepared in distilled water and stored at 4°C. Plasma containing known concentrations of baclofen were prepared by appropriately diluting the stock solution with drug-free plasma. These plasma standards were stored frozen at −20°C and made fresh each week. The plasma standards were then used to standardize the extraction procedure and to calibrate the HPLC determination. The concentration of baclofen in plasma samples was determined by measuring peak height and using a calibration plot determined with the plasma standards.

Extraction recoveries

The recovery of baclofen extracted from plasma with the Bond-Elut columns was estimated by comparing the results obtained with a non-extracted standard at the same concentration in the phosphate—acetonitrile (75:25) buffer used to elute the baclofen from the Bond-Elut columns. The recoveries were determined from the mean of six replicates.

Screening for interfering drugs

A range of drugs that are commonly co-administered with baclofen were screened for their possible interference in the assay. Plasma from patients known to be taking a particular drug was processed by the method and the resultant HPLC profile checked for interfering peaks at the retention time of baclofen. This process eliminated any potential interference from the parent

drug and its metabolites at levels likely to be encountered in the clinical situation.

Baclofen plasma levels

Blood samples were taken from multiple sclerosis patients on chronic baclofen therapy by venipuncture at hourly intervals (for 5 h) starting 1 h after the morning dose. The blood samples were placed in heparinised tubes and centrifuged immediately to obtain the plasma which was stored frozen at −20°C until assayed.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms obtained from plasma spiked with a known amount of baclofen (A), drug-free plasma (B), and plasma from a patient after a normal oral dose of baclofen (C). Under the HPLC conditions used baclofen had a retention time of 9.4 min. No interference was observed from any endogenous plasma component, although the chromatogram obtained with the drug-free plasma contained several peaks. It was not possible to remove these endogenous plasma components from the Bond-Elut column without suffering further losses of baclofen. The recovery of baclofen from plasma was found to be 38.7 ± 1.4% (n = 6) at 50 ng/ml and 46.0 ± 0.9% (n = 6) at 1000 ng/ml. These recoveries are comparable to those reported for the gas chromatographic methods [2]. No baclofen could be detected in plasma collected after passing through the Bond-Elut column and reextracted. Therefore, the relatively poor recoveries are a result of the clean-up washes of the Bond-Elut column rather than poor initial extraction of baclofen by the Bond-Elut column.

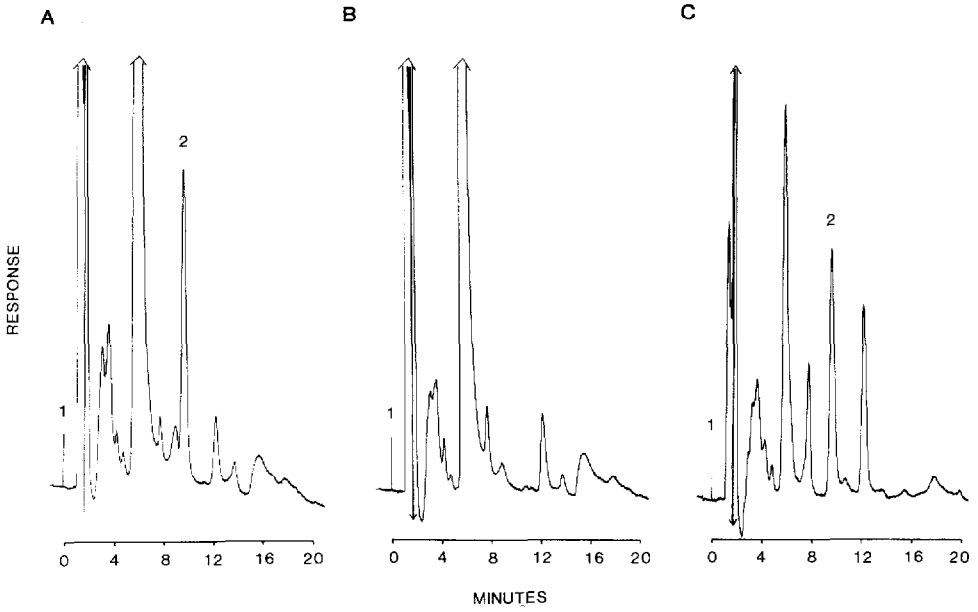

Fig. 1. HPLC profiles of (A) drug-free plasma spiked with 800 ng/ml baclofen, (B) drug-free plasma, (C) patient plasma sample (725 ng/ml baclofen) collected 1 h after a dose of 90 mg baclofen. 1 = Injection site; 2 = baclofen.

The intra-assay precision of the method was determined from replicate assays (n = 6) of drug-free plasma spiked with known concentrations of baclofen. A coefficient of variation (C.V.) of 2.0% at 1000 ng/ml and 3.7% at 50 ng/ml was obtained. This is an improvement over C.V. values of up to 10% obtained with previously published methods [1—4]. The relatively low C.V. obtained for the method reported here makes the use of an internal

TABLE I

DRUGS SHOWING NO INTERFERENCE WITH BACLOFEN DETERMINATION

Anticonvulsants	*Tranquilisers*	*Antibiotics*
Phenytoin	Phenobarbitone	Amikacin
Carbamazepine	Oxazepam	Doxycycline
Primidone	Chlordiazepoxide	Rifampicin
Valproate	Haloperidol	Penicillin
Clonazepam	Thioridazine	Septrin
	Amylobarbitone	Gentamicin
		Mandelamine
Antidepressants	*Sedatives*	*Analgesics*
Doxepin	Nitrazepam	Doloxene
Amitriptyline	Temazepam	Aspirin
Nortriptyline	Chloral hydrate	Paracetamol
Imipramine	Lorazepam	Naprosyn
Antispasticity	*Antinauseants*	*Anticholinergics*
Diazepam	Prochlorperazine	Propantheline
Orphenedrine	Metoclopramide	

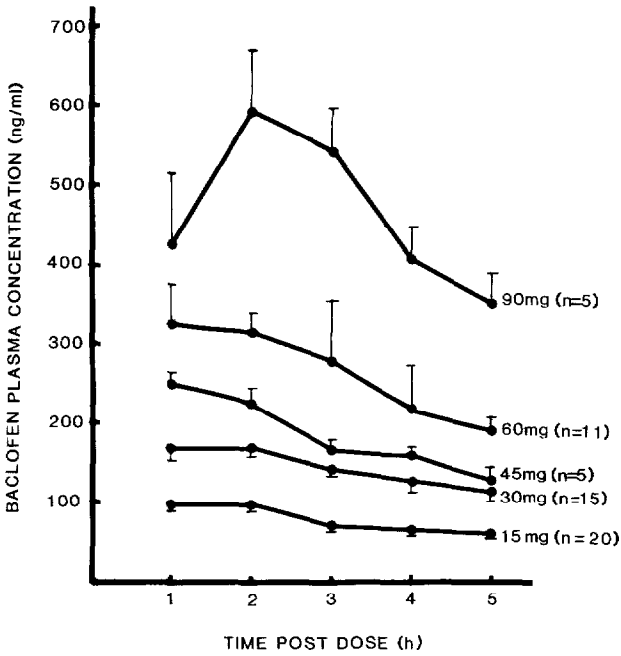

Fig. 2. Mean (± S.E.M.) baclofen plasma levels in multiple sclerosis patients on chronic baclofen therapy.

standard unnecessary despite the low recoveries obtained. This is perhaps fortunate as it would be difficult to find a suitable compound with similar properties to baclofen and not suffer any interference from endogenous plasma components.

The assay was linear over the plasma concentration range 0.05—1.0 μg/ml ($y = 0.187x$, $r = 0.997$, $n = 7$) which spans the accepted therapeutic range [5]. No interference was observed with plasma from patients known to be taking drugs that are commonly co-administered with baclofen (Table I). Possible interference may be observed with flucloxacillin and amoxycillin.

As an example of the use of the method Fig. 2 shows the mean baclofen plasma levels in multiple sclerosis patients on chronic therapy at a range of doses. The difficult and previously lengthy methods for the determination of baclofen has meant that this information in so many patients has not been available before.

In summary, the method described here offers quick and straightforward sample preparation giving a total assay time of 15 min compared to up to 2 h for the extraction alone as in a previously published method [2]. The use of commonly available HPLC equipment rather than the less common gas chromatograph—mass spectrometer overcomes the need for derivatisation and makes the method widely accessible.

ACKNOWLEDGEMENTS

We wish to gratefully acknowledge the support and funds made available from the Multiple Sclerosis Society of Victoria, Ciba-Geigy (Australia), and the Whole-Time Medical Specialists Fund, Alfred Hospital.

REFERENCES

1 E. Knutsson, U. Lindblom and A. Martensson, J. Neurol. Sci., 23 (1974) 473.
2 P.H. Degen and W. Riess, J. Chromatogr., 117 (1976) 399.
3 G. Eriksson and C.-G. Swahn, Scand. J. Clin. Lab. Invest., 41 (1981) 185.
4 C.-G. Swahn, H. Beving and G. Sedvall, J. Chromatogr., 162 (1979) 433.
5 G.K. McEvoy, Drug Information, American Hospital Formulary Service, Amer. Soc. Hosp. Pharm., Bethesda, MD, 1984, p. 407.