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COMBINED USE OF AN AUTOMATED SAMPLE PROCESSOR AND A POLYMER-BASED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMN TO DETERMINE THE PHARMACOKINETICS OF LABETALOL IN MAN

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SUMMARY

An improved high-performance liquid chromatographic (HPLC) assay has been developed for the analysis of labetalol in human plasma. The method is based on the combined use of an automated sample processor, reversed-phase analysis on a microparticulate polymer-based HPLC column and fluorescence detection. The pH stability of the polymeric column packing material allowed the use of a mobile phase adjusted to pH 9.5, which was optimal for the fluorescence of labetalol.

Assay validation was undertaken over the labetalol concentration range 2–100 ng/ml. Calibration curves were essentially linear, and the mean coefficient of variation was 5.3%. The assay has been used for the analysis of clinical samples in support of pharmacokinetic studies.

INTRODUCTION

Labetalol (Benzamide, 2-hydroxy-5-{1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl}-monohydrochloride), a combined peripheral β-adrenergic and α_1-receptor antagonist, is used in the treatment of hypertension. Labetalol is rapidly absorbed following oral administration; linear correlations have been found between the dose administered, the concentration of labetalol in the plasma, and the decrease in standing blood pressure[1]. Analytical methods for the analysis of labetalol in plasma are necessary for monitoring patient compliance and also for the study of the pharmacokinetics of labetalol in healthy volunteers and patients suffering from renal or hepatic disease[2,3].

Labetalol was first determined in plasma by measurement of the fluorescence of an ammoniacal aqueous solution, following solvent extraction into chloroform[4]. The basification of the extract was required to maximise the fluorescence of labetalol; this method was suitable for the analysis of the drug in 1 ml plasma down to 20 ng/ml. It was later established that labetalol is subject to extensive first-pass metabolism[4,5]; the peak plasma concentration after a 200-mg dose is *ca.* 100 ng/ml. The elimination half-life is *ca.* 3–6 h. In order to determine the pharmacokinetics of la-

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betalol, an assay is required which is sufficiently sensitive to measure the drug down to 4 ng/ml or less, particularly at doses lower than 200 mg.

High-performance liquid chromatographic (HPLC) methods based on UV detection have been developed for the routine analysis of clinical samples, but for those methods cited[6,7], precision data were lacking for labetalol concentrations below 50 ng/ml. Further improvements were made by the use of UV detection at 205 nm. This allowed the measurement of the drug at concentrations of 10–100 ng/ml with a coefficient of variation (C.V.) of 4–6%[8]. HPLC analytical sensitivity may be further improved by the use of fluorescence detection. The enhanced fluorescence of labetalol at high pH has prompted the use of post-column addition of base in order to achieve higher sensitivity[9]. However, plasma samples containing labetalol at less than 8 ng/ml were not measured.

Recently, a new class of macroporous copolymeric chromatographic packing materials [PRP-1 (Hamilton Bonaduz, Bonaduz, Switzerland), PLRP-S] has been developed. This has facilitated further improvements in the analytical detection of labetalol. The pH stability of these materials allows the use of a mobile phase at high pH. This obviates the need for post-column addition of base to achieve maximum fluorescence. Using a column (PRP-1) containing 8- to 12-μm spherical, macroporous copolymer particles, good sensitivity has been achieved, although labour-intensive solvent extraction stages for sample preparation and an internal standard were required[10].

The procedure described in this paper is based on semi-automated sample preparation with the Advanced Analytical Sample Processor (AASP) and fluorescence detection and utilises a 5-μm copolymer-based HPLC column (PLRP-S) in conjunction with an alkaline mobile phase. This method allows the selective determination of labetalol down to 2 ng/ml, in only 500 μl plasma. Good precision is obtained without the use of an internal standard.

EXPERIMENTAL

Materials

Labetalol hydrochloride was supplied by Glaxo Central Analytical Services Department (Barnard Castle, U.K.); ammonium carbonate, sodium hydroxide (both AnalaR grade), and ammonia solution (Aristar, 35%) by BDH (Poole, U.K.); acetonitrile and methanol (both HPLC grade) by Rathburn Chemicals (Walkerburn, U.K.); dichloromethylsilane by Aldrich (Gillingham, U.K.); control human plasma by the Human Pharmacology Unit (Glaxo Group Research, Ware, U.K.).

Instrumentation

The HPLC system comprised a Model 8700 solvent delivery system (Spectra-Physics, St. Albans, U.K.), a Spectroflow 400 pump (Severn Analytical, Gloucester, U.K.), an Advanced Analytical Sample Processor (Jones Chromatography, Llandbradach, U.K.), a RC 200 fluorescence detector (Baird Atomic, Braintree, U.K.), a PLRP-S column (15 × 0.46 cm, I.D., 5-μm polystyrene–divinylbenzene packing) (Polymer Labs., Church Stretton, U.K.), and a Sigma Electronic Noise Filter (Magnus Data, Aylesbury, U.K.). Data handling was carried out with a Trivector Trojan data system (Trivector Systems International, Sandy, U.K.).

Preparation of standards

Plasma required for the calibration curve standards (spiked with labetalol at 2, 5, 10, 25, 50 and 100 ng/ml) and the quality control samples (spiked with labetalol at 4.5, 30.0 and 75.0 ng/ml) was prepared by mixing 0.2-ml aliquots of aqueous labetalol with 1.8 ml plasma (or multiples thereof). Each batch of quality control plasma was divided into 2-ml aliquots and stored frozen (−18°C) until required. Control plasma was prepared by mixing 0.2 ml distilled water with 1.8 ml human plasma (or multiples thereof).

Extraction procedure

The extraction of labetalol from human plasma was carried out by the elution of the sample through AASP cassettes (the cartridges of which contained a chromatographic packing material (C_{18}) which retained the drug) prior to the on-line elution of the drug into the chromatographic system. Before sample loading, the AASP cassettes were conditioned (using the off-line Vac Elut module at 25 p.s.i.) with 1.0 ml methanol, followed by 2.0 ml distilled water. The plasma samples (0.5 ml) were vortex-mixed (in disposable polypropylene test tubes) with 0.5 ml 0.02 *M* sodium hydroxide (final pH, 10.5) and eluted through the cassette. The cassettes were finally washed by eluting each cartridge with 1.0 ml distilled water.

Chromatographic analysis

Chromatographic analysis was carried out (at ambient temperature) using a PLRP-S column, a mobile phase of ammonium carbonate (0.05 *M*, adjusted to pH

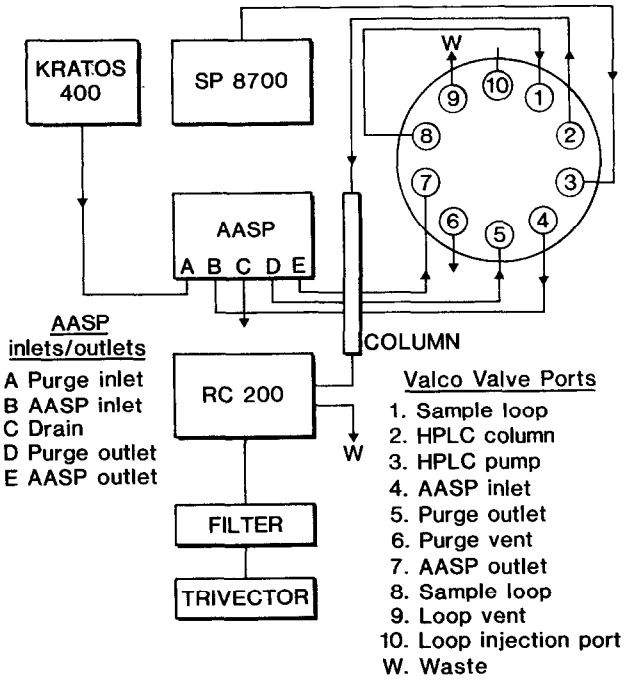

Fig. 1. The chromatographic system used for the analysis of labetalol in human plasma.

9.5 with 35% ammonia solution) and acetonitrile in the ratio 7:3 (flow-rate, 1.2 ml/min) and fluorescence detection (excitation wavelength, 334 nm; emission wavelength, 412 nm). The chromatographic separation took 8 min. Prior to sample injection, the AASP cartridges were purged with distilled water (to exclude trapped air) by the use of the AASP purge function. As a result of the inadequate pumping characteristics of the AASP internal pump, which was a feature of the early AASP models, distilled water was pumped (at 0.5 ml/min for the entire duration of the analysis) directly into the purge pump inlet by an external pump (Kratos Spectroflow 400). A schematic representation of the analytical system is given in Fig. 1. Labetalol was quantified by the external standard method based on peak area.

Assay validation

Assay validation was undertaken by regression analysis of calibration curves obtained on four separate days, by determination of the within-batch and between-batch C.V., and by the development of a chromatographic system showing good resolution of labetalol from endogenous contaminants. The within-batch C.V. was determined by repeat analysis of seven calibration curve standards (at 0, 2, 5, 10, 25, 50 and 100 ng/ml) in six consecutive batches over a 14-h period (the approximate period of time for an analytical run during the analysis of study samples). The between-batch C.V. was determined by the repeat analysis (in triplicate) of plasma quality control samples (spiked with labetalol at 4.5, 30.0 and 75.0 ng/ml) relative to a standard calibration curve on three separate days.

Analysis of study samples

Plasma samples obtained from pharmacokinetic studies of labetalol were analysed in daily batches of approximately 80 samples. This number comprised fourteen calibration curve standards (duplicate analysis at 0, 2, 5, 10, 25, 50 and 100 ng/ml) and nine quality control samples (triplicate analyses at 4.5, 30.0 and 75.0 ng/ml), the remainder being study plasma samples.

RESULTS AND DISCUSSION

Results obtained from the assay validation studies and from the application of the assay to the analysis of volunteer samples have confirmed the suitability of the assay for the measurement of labetalol in human plasma over the concentration range 2–100 ng/ml.

The AASP solid-phase extraction system proved to be both robust and easily used, allowing the preparation of 70–80 samples in 2–3 h. The extraction recovery of labetalol from human plasma ranged from 90–100%. This approach to sample preparation for the analysis of labetalol represents a marked improvement on previously cited methods based on complex and time-consuming liquid–liquid extraction[9–11].

Reversed-phase chromatography on 5-μm polystyrene–divinylbenzene (PLRP-S) produced good separation of labetalol from endogenous contaminants and the use of a mobile phase adjusted to pH 9.5 allowed maximal analytical sensitivity without deleterious effects on the quality of the chromatograms (Fig. 2). The retention time of labetalol under these conditions ranged from 4–5 min and showed no

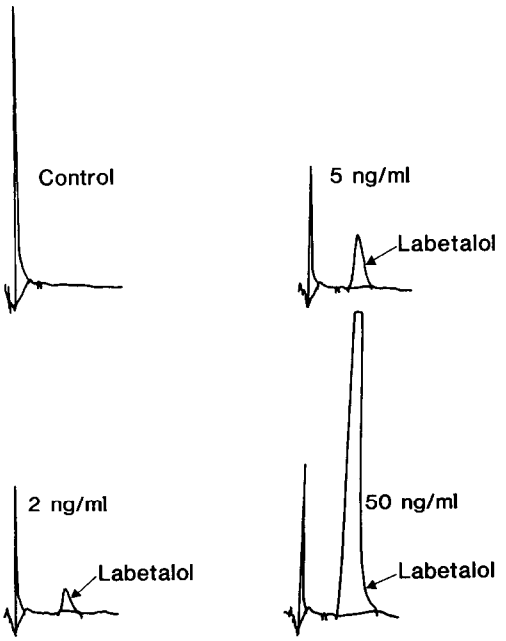

Fig. 2. Chromatograms obtained following the analysis of labetalol in human plasma.

significant change over a period of four months. The chromatographic peak-shape of labetalol, though symmetrical, was broader than usual for silica-based reversed-phase columns[7]. This observation is consistent with chromatograms of labetalol on 10-μm polystyrene–divinylbenzene (PRP-1)[10]. Under these conditions, labetalol was readily quantified down to 2 ng/ml from 500 μl plasma.

A plot of the peak area of labetalol *vs.* labetalol plasma concentration (at 0, 2, 5, 10, 25, 50 and 100 ng/ml) was linear ($r > 0.999$). The within-batch quantitative precision (C.V.) of the assay, calculated from six replicate analyses of calibration standards in a single analytical run, ranged from 1.5 to 11.5% (mean 5.3%). The between-batch quantitative precision of the assay was determined by the triplicate analysis of quality control samples (spiked with labetalol at 4.5, 30 and 75 ng/ml) in conjunction with plasma samples from twelve healthy volunteers over a period of eight weeks. The results obtained from the analysis of these samples were 4.2 ± 0.9, 29 ± 2.4 and 73 ± 4.3 ng/ml, respectively. In addition, it was found that the assay did not require an internal standard. This both simplified the analytical methodology and reduced analysis time to 8 min/sample.

This assay was successfully used for the analysis of human plasma samples from a volunteer study of the pharmacokinetics of labetalol administered in three different (200 mg) tablet formulations. The assay was sufficiently sensitive to allow the measurement of labetalol in the plasma up to 24 h after administration. The plasma labetalol concentration–time profile and the associated model-independent pharmacokinetic parameters [C_{max}, T_{max}, $t_{½}$ (elimination), and area under the curve (AUC) (0, ∞)], obtained for one of these formulations (mean profile from twelve

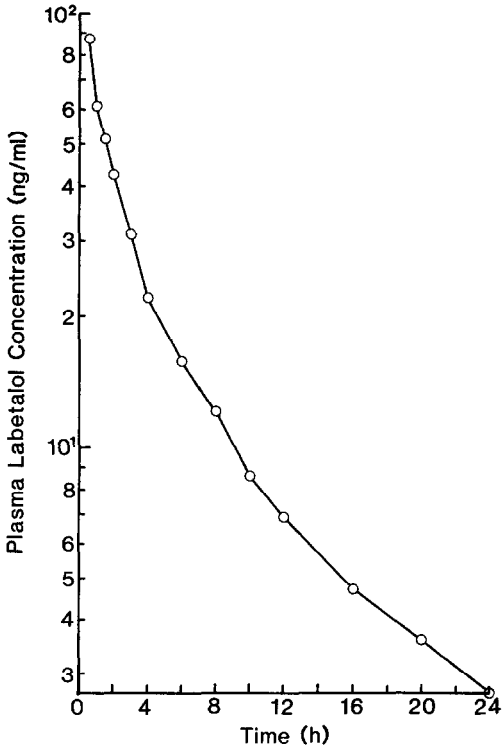

Fig. 3. A plasma labetalol concentration time profile, obtained following the administration of a 200-mg labetalol tablet formulation to healthy volunteers. Each time-point is the mean of twelve volunteers. AUC (0, ∞): 346.11 ng/ml/h, $t_{½}$: 5.18 h, C_{max}: 87.30 ng.ml^{-1}, T_{max}: 0.5 h.

volunteers), is presented in Fig. 3. These findings compare well with previous studies[4,5,12], which have shown that labetalol is rapidly absorbed and extensively metabolised after an oral dose. The terminal half-life of 5.18 h, determined in the present study, is also consistent with previously cited values of 3–6 h[12,13].

CONCLUSIONS

The pH stability of reversed-phase columns based on 5-μm polystyrene–divinylbenzene allows the use of an alkaline mobile phase without deleterious effects on the chromatogram of labetalol. This approach can be readily used to increase the analytical sentivity for labetalol by fluorescence detection, as this drug exhibits maximal fluorescence at high pH. When this chromatographic system is combined with the AASP extraction module, labetalol can be readily analysed in plasma over the concentration range 2–100 ng/ml. The assay procedure described is more sensitive and more robust than previously cited methods, and the short analysis time allows a high daily throughput of samples.

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