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The assay and resolution of the beta-blocker atenolol from its related impurities in a tablet pharmaceutical dosage form

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Abstract: For the complete resolution and determination of the β-blocker atenolol and its manufacturing impurities a high-performance liquid chromatographic method is developed using structured optimization techniques. The method utilizes a 300 × 3.9 mm i.d. stainless steel column packed with μBondapak C_{18} and a mobile phase containing methanol–25 mM potassium dihydrogen orthophosphate containing 0.06% (v/v) dibutylamine (20:80, v/v) (pH 3.0). The flow rate of 1.0 ml min^{-1} is used and a detection wavelength of 226 nm. The linearity and repeatability are good for the present compound over the range 1.5–510.0 μg ml^{-1} ($r > 0.99$ and RSD 0.27%, $n = 10$). Application of the method to 50 and 100 mg tablets gave recoveries of 99% (w/w) and reproducibilities of (RSD) 1.1 and 0.52%, respectively ($n = 5$). The manufacturing impurities are found to be >0.5% (w/w) of the atenolol peak.

Keywords: *Liquid chromatography; β-blocker atenolol; manufacturing impurities; solvent selectivity; modified simplex optimization; method validation.*

Introduction

Drugs inevitably possess manufacturing impurities derived from the synthetic route and with ageing there is the possibility of other impurities being formed, where all or some of these impurities may be toxic. In developing suitable stability indicating and routine chromatographic methods of analysis the aim is to resolve such impurities from the parent compound in order that accurate potency determinations can be made. The impurities may need to be synthesized, be obtained as pure substances or as mixtures from the manufacturer or other commercial suppliers or may be available as standard reference materials.

In the development of a suitable high-performance liquid chromatographic (HPLC) method, the resolution of the components can most readily be facilitated by consideration of the mobile phase composition and in this respect it is beneficial to apply structured procedures to optimize the mobile phase composition.

One example where optimization techniques could prove beneficial is in the resolution of impurities from the drug atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy)phenyl acetamide, which is one of a number of drugs collectively known as β-blockers. Its pharmaceutical use is in the management of hypotension, angina pectoris, cardiac dysrhythmias and myocardial infarction, where it acts preferentially upon the β-adrenergic receptors in the heart [1]

On examination, the literature is deficient in suitable routine methods for potency and impurity determinations of atenolol in both the bulk drug and solid dosage formulations. Monographs for atenolol and atenolol tablets are published in the British Pharmacopoeia (BP 1988) where UV spectroscopy is recommended for the analysis of tablets and a nonaqueous titration for determination of the bulk drug. In addition, for the impurity analysis an ion-pair RP-HPLC method is described. Unfortunately this method suffers from a major disadvantage in that no visible resolution is exhibited between the peaks of two of the impurities and there is poor resolution between another and the atenolol peak. The method also, when applied in the authors' laboratory, displayed poor reproducibility and was associated rapid column degeneration. In

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the present work an optimized and fully validated method of analysis is described which enables the accurate determination of both the drug and related impurities in bulk drug and solid dosage formulation, by utilizing one RP-HPLC method.

Atenolol possesses a number of manufacturing impurities which are generally detected in the bulk drug and inevitably therefore, in the finished dosage product. There are four main impurities, namely 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetic acid(I), *p*-hydroxyphenyl acetamide(II), *p*-2,3-dihydroxypropoxyphenylacetamide(III) and *p,p*[*N*-isopropyl-3,3-imino-bis(2-hydroxy-propoxy) bis(phenylacetamide)(IV). A number of very minor impurities also have been reported [2]. These major impurities fortunately are available within an analytical impurity mixture standard obtainable from the BP, and also are obtainable from the manufacturers of atenolol.

Experimental

The chromatographic system consisted of a dual reciprocating pump (Model 420, Kontron, Milan, Italy) and low pressure gradient former (Model 325, Kontron) connected to a dual variable wavelength UV detector (Model 430, Kontron). The HPLC system was interfaced with an IBM-AT computer/data station and controlled through resident Kontron multitasking software which allowed post-data analysis whilst allowing further on-line acquisition of data.

Chromatographic conditions

A 300 × 3.9 mm i.d. stainless steel column packed with μBondapak C_{18} (Waters Associates, Millipore, Peterborough, UK) was used with a mobile phase containing methanol–25 mM potassium dihydrogen orthophosphate containing 0.06% (v/v) dibutylamine (20:80, v/v) (pH 3.0). This mobile phase was used for both the determination of the related impurities and in the potency of the drug. The flow rate was 1.0 ml min^{-1} and the detection wavelength was 226 nm at 0.05 aufs. A linear gradient was used and the organic modifier concentration was decreased from 90 to 10% over a period of 15 min.

Reagents and materials

Atenolol and atenolol tablets (50 and 100 mg) were obtained from APS/Berk Ltd (a subsidiary of Rhône Poulenc Rorer, Dagenham, Essex, UK) as was the HPLC grade methanol (Rhône Poulenc Rorer). Potassium dihydrogen orthophosphate was analar grade and also supplied by Rhône Poulenc Rorer. Dibutylamine was supplied by Aldrich Chemicals. The samples of the BPCRS impurity standard and pure drug (100%) were obtained from the B.P. (British Pharmacopoeia Commission Laboratories, Middlesex, UK).

Atenolol and impurity extraction from tablets

For the assay of 50 and 100 mg tablets, 100 tablets were weighed, powdered and ground. The weight equivalent to 100 mg atenolol was accurately weighed into a 100 ml volumetric flask, extracted with HPLC methanol filtered or centrifuged and made up to volume. To 5 ml of the supernatant in a 100 ml volumetric flask was added 15 ml of methanol and the solution was diluted to volume with the aqueous portion of the mobile phase to obtain a final drug concentration of 0.05 mg ml^{-1} atenolol.

The standard solution for the assay was prepared by accurately weighing atenolol (100 mg) into a 100 ml volumetric flask and dissolving in methanol. To 5 ml of this solution was added 15 ml of methanol and the solution made to volume with the aqueous portion of the mobile phase, again to obtain a final concentration of 0.05 mg ml^{-1}.

For the impurity examination of the bulk drug and dosage form, the equivalent of 100 mg of atenolol from the powdered tablets was weighed into a 100 ml volumetric flask and extracted with 20 ml methanol, made up to volume with buffer and then mixed and filtered. The filtrate was then diluted (1:200) with mobile phase to provide an external standard for the impurity limitation. At an early stage, in order to determine the retention times of the BP impurities, 60 mg of the BP impurity standard was weighed into a 100 ml volumetric flask and dissolved in mobile phase.

Procedure

For the assay of the requisite sample, 10 or 20 μl was injected onto the column with the samples bracketed between standards. From the resultant peak area integrals, a mg per tablet content of atenolol was determined. Alternatively if assaying the bulk drug, a percentage purity was calculated. For the impurity check on the atenolol tablet samples,

10 μl of the diluted (1:200) solution, prepared from a concentrated sample solution containing 1 mg ml^{-1} of atenolol in mobile phase, was injected and this injection was followed by the concentrated sample solution (1 mg ml^{-1}). The BP standard impurity solution was then injected, whilst noting carefully the retention characteristics of the major impurities as reference points. Secondary peak areas other than that from the peak due to atenolol were noted. In addition these peaks were compared with the retention times of the manufacturing impurities exhibited within the chromatogram of the BP impurity standard solution and from this a total impurity level was calculated by comparison with the diluted sample solution of atenolol. Here the total sum of the peak areas of the impurities in the concentrated sample solution (equivalent to 1 mg ml^{-1} atenolol) were divided into the peak area of the diluted sample solution and the ratio then multiplied by 0.5 in order to obtain a w/w per cent total impurity level.

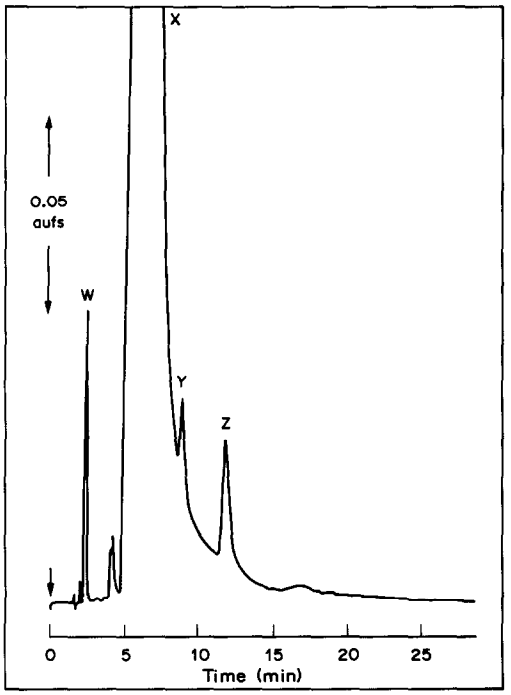

Figure 1
Partial resolution of atenolol from its major manufacturing impurities using the method of the British Pharmacopoeia (Vol. 1, 1988, p. 49). The chromatographic conditions were: 200 × 4.9 mm i.d. stainless steel column packed with 5 μm Hypersil-ODS. The mobile phase used was methanol–aqueous 0.5 M sodium dodecyl sulphate (60:40, v/v) (pH 3.0). The flow rate was 1.5 ml min^{-1} and the detection wavelength 226 nm at 0.05 aufs.

Results and Discussion

In order to establish the potency of atenolol tablets, the British Pharmacopoeia [3] describes a spectrophotometric method. However, as the current production of this tablet dosage form (50 and 100 mg) includes tablet film coating, interference by non-specific absorption of the coloured coating occurs, which is not eliminated by filtration and dilution during sample preparation. On these grounds the spectrophotometric method is considered to be unsuitable. In contrast, the BP [3] describes an ion-pair RP-HPLC method for assay of the related impurities. When this method was applied in our laboratory (Fig. 1) incomplete resolution of the related impurities was observed and PPA and Diol were found to coelute. Additionally, the tertiary amine impurity peak is incompletely resolved from the peak due to atenolol eluting on the down-slope of the atenolol peak, which leads to problems in determination and reduces the overall sensitivity of detection of the method. Other disadvantages of the method include the analysis time which is consistently in excess of 25 min and the instability of the alkyl-bonded silica packing material under the conditions of operation.

The purpose of the present project was to improve upon the BP method by a systematic consideration of the effects of column type, organic modifier, pH and ion-pairing agent type and concentration upon the reliability of the assay. In order to optimize some of these variables the structured procedure of method design was examined through the simultaneous solvent selectivity approach [4–7] and sequential modified simplex design [8].

In initial HPLC experiments the column type used was that described in the BP method; namely a 250 × 4.6 mm i.d. stainless steel column packed with 5 μm Hypersil ODS packing material (Hichrom, Reading, UK). As a starting point a gradient experiment was carried out using methanol and 25 mM potassium dihydrogen orthophosphate buffer (pH 3.0) with dibutylamine as ion pairing agent. The linear gradient was progressed at 6.66% min^{-1} over a period of 15 min beginning with 90% methanol to 90% buffer and injecting 20 μl of a mixture containing all the major impurities and atenolol. From the gradient chromatogram the optimal composition was

found to be methanol–25 mM potassium dihydrogen orthophosphate and dibutylamine (0.06%, v/v) (20:80, v/v) (pH 3.0) and this was further fine-tuned in the isocratic mode. However, resolution of the peaks of the components from the BP reference impurity standard was not satisfactory and the column was replaced with a 10 μm μBondapak ODS packing (300 × 3.9 mm i.d. stainless steel column). This column exhibited improved peak symmetries and demonstrated greater stability at low mobile phase pH for repeat sample injections. It is interesting to note that the best gradient mobile phase composition was also suitable for this column.

Starting from the above composition further optimization experiments were carried out. Isoeluotropic mixtures containing acetonitrile, methanol and tetrahydrofuran were examined. The proportions were calculated using an average value, obtained from the Snyder equation [4]: $ST = sa\phi a + sb\phi b + sc\phi c \ldots$, etc., where s is the solvent strength weighting factor which is: 2.6 for methanol, 3.2 for acetonitrile, 4.5 for tetrahydrofuran, and 0 for water. ϕ is the volume fraction of the organic phase. ST is the solvent strength for the required organic phase and the Schoenmaker equation: %ACN (v/v) = 0.32ϕ MeOH + 0.57ϕ MeOH; %THF (v/v) = 0.66ϕ MeOH, where ϕ = volume fraction of modifier. Both equations resulted in errors in the determination of the solvent proportions and since the values obtained from the two equations were only marginally different it was considered that an average value could reduce or eliminate the error. The purpose of examining these isoeluotropic mixtures was to observe the selectivity afforded by each mobile phase on the separation, whilst retaining very similar retention times. From the triangle method (involving 10 experiments) the measurement criterion was based on peak resolution (Rs). It was observed that both acetonitrile and tetrahydrofuran mixtures gave reduced resolution of the impurities from the atenolol peak and compromised the peak shape quality of the impurities in contrast to the methanol–buffer mixture. In the case of dual organic modifiers and when mixing all modifiers (33.33%, v/v against the buffer), coelution of the component peaks was observed. Therefore from these experiments a mobile phase based on methanol and containing 25 mM potassium dihydrogen orthophosphate + 0.06% (v/v) dibutylamine (20:80, v/v) (pH 3.0) was found to give the best Rs values for the worst separated peak pair (Rs 2.0).

In contrast to the more structured approach adapted to organic modifier variation, a univariate approach was applied to adjustment of pH. Over the pH range 3–7, an increase in pH was accompanied by a decrease in resolution. Specifically, at a pH greater than 4, peak overlap of atenolol with impurity I and II with III was observed. Additionally, it was observed that at pH 5, peak splitting of the tertiary amine peak did not occur since only one ionic form was then present. This was not the case at a pH above 5.0 where two species were present, having slightly different retention times and as a result peak splitting occurs. However this impurity had shown a substantial increase in retention time. Thus a pH of 3.0 was used in the experimental work, which after continued use on this column packing, apparently does not compromise its stability, and still gives good chromatographic response. The reason for this stability difference over the Hypersil ODS column packing in the BP method was not completely clear, but it is considered that the nature of the ion-pairing component is also important.

In this early work the ion-pairing agent was dibutylamine which apparently acted as an end-capping agent for the residual silanols and as an ion-pairing agent. To assess the effect of the ion-pairing agent on the separation, a set of experiments was then carried out with different alkyl chain length amines, namely dimethylamine, dipropylamine, dibutylamine, dipentylamine and dioctylamine and a related dibenzylamine. In turn each was added to the mobile phase with individual correction for percentage concentration. In these experiments it was observed that from dimethylamine to dibutylamine increased Rs-values for the impurity separations (Fig. 2) occurred, but above dibutylamine the differences in Rs-value decreased considerably and could be considered as negligible, which indicated that the original choice of ion-pair agent had been fortuitous.

Finally in the optimization strategy a recheck was made on the modifier composition through the use of the modified simplex procedure. As a criterion for measuring the response during this method a reduced chromatographic response function (CRF) was adopted, as defined by the expression:

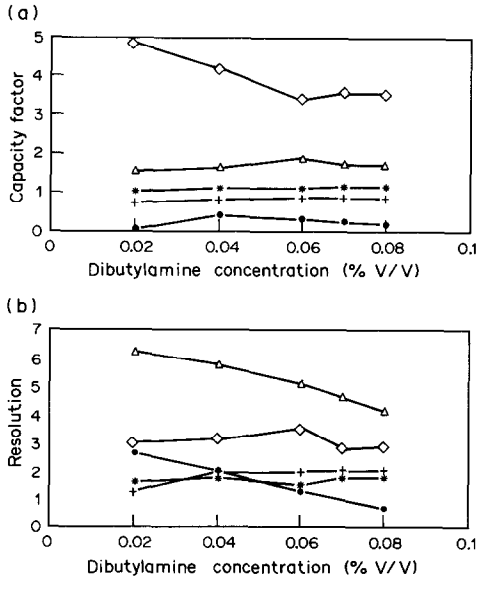

Figure 2
(a) Effect of dibutylamine on resolution of the impurities of atenolol (resolution vs percentage dibutylamine). (b) Effect of dibutylamine on resolution of the impurities of atenolol (capacity factor vs percentage dibutylamine).

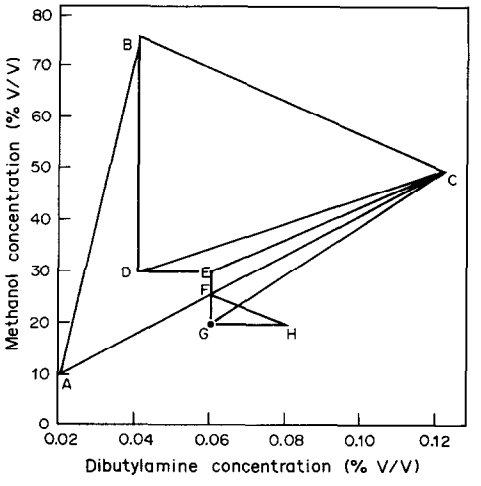

Figure 3
Modified simplex diagram for the resolution of the manufacturing impurities of atenolol. Points A–G represent simplexes which move from the initial simplex ABC to the final simplex FGH where the optimum lies at G through expansions and contractions, following the CRF value. Together these operations provide the means for the simplex figures to expand and accelerate toward the optimum region and having approximately located this region, to contract and reduce the search region until the optimum is located precisely.

$$\text{CRF} = \text{Rs} + n(T_1 - T_2),$$

where Rs is the resolution between the least separated pair of peaks, n is the number of observed eluted peaks, T_1 is the last peak retention and T_2 the required analysis time [9].

The optimization was based on two variables, namely organic modifier and dibutylamine concentrations. The experimental programme development was guided through improvement in CRF by varying the modifier composition through inward reflection from the initial simplex towards the optimum for maximum resolution of the impurities; the optimum lies at point G in Fig. 3. As an extension of the optimization experiments, the homogeneity of the atenolol peak also was assessed in the pharmaceutical preparation by capturing and comparing spectra throughout the elution profile. This was reinforced by the additional measurement of wavelength ratios at two selected wavelengths as a further check of chromatographic peak purity. Unfortunately, it was not possible to perform the same tests on the impurities present due to their low concentrations in the bulk drug. The optimum chromatographic parameters therefore were found to be methanol–25 mM potassium dihydrogen orthophosphate containing 0.06% (v/v) dibutylamine (pH 3.0) adjusted with 100% orthophosphoric acid (20:80, v/v) (pH 3.0). This was on a column of 300 × 3.9 mm i.d. μBondapak C18; flow rate was 1 ml min^{-1} at a detection wavelength of 226 nm at 0.05 aufs.

In validating the assay method (Fig. 4) the linear range of response for atenolol as defined by the expression: $y = 23.62x + 0.015$; ($R = 0.999$, $n = 10$) at the chosen wavelength of 226 nm (selected to increase the sensitivity of detection of the impurities) was narrow (1.5–510 μg per ml in mobile phase). Nevertheless it was found to be sufficiently large to enable accurate potency determinations. This was obtained through preparing placebo mixtures of the formulation excipients and adding accurately known quantities of atenolol equivalent to 40–200% (w/w) of the nominal assay concentration of 0.05 mg ml^{-1}. It was calculated that at 40% the recovery of the system was 100.3% (w/w), at 100.0% the recovery was 99.4% (w/w) and at 200.0% it was observed that a recovery of 98.61% (w/w) was obtained. In order to test repeatability a standard solution (0.05 mg ml^{-1}) was injected several times and the RSD value for replicate injections was 0.27% ($n = 12$). Day-to-day reproducibility was also excellent (RSD 0.19%). Retention times and resolution also were

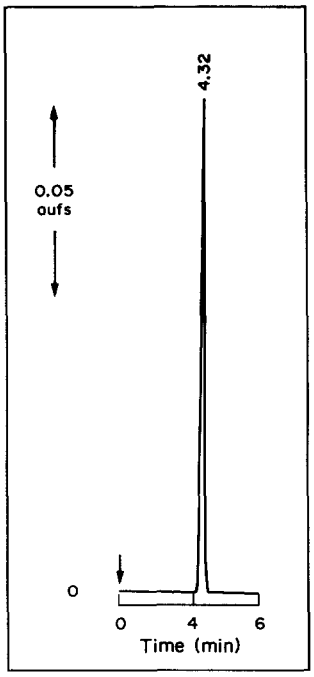

Figure 4
Typical assay chromatogram for the determination of the potency of atenolol in the bulk drug and solid dose formulation using the newly developed HPLC assay. The chromatogram represents a 0.05 mg ml^{-1} of atenolol in mobile phase. The chromatographic conditions were: 300 × 3.9 mm i.d. stainless steel column packed with μBondapak and a mobile phase of methanol–25 mM potassium dihydrogen orthophosphate (20:80, v/v) containing 0.06% (v/v) dibutylamine (pH 3.0). The flow rate was 1.0 ml min^{-1} at a detection wavelength of 226 nm at 0.05 aufs.

observed to be highly reproducible and not affected by slight changes in mobile phase composition.

Application of the method to two batches of 50 and 100 mg tablets which were ground and powdered and then subjected to replicate assays gave a mean assay value of 49.2 and 97.6 mg, respectively and a RSD of 1.1% (n = 10) and 0.5% (n = 5), respectively.

In conclusion the chromatographic method developed resolves the major manufacturing impurities from atenolol (Fig. 5) and allows for these impurities to be determined accurately, and the potency of atenolol in the bulk drug or tablets to be assessed. The method is both easy to perform, accurate and reproducible and

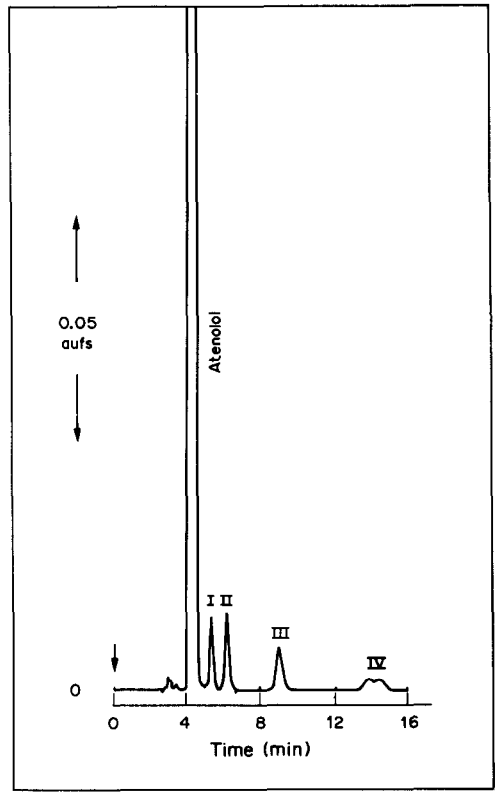

Figure 5
Resolution of the major manufacturing impurities of atenolol from a BP impurity standard solution (0.6 mg ml^{-1}). For chromatographic conditions see Fig. 4.

therefore could be used for routine analysis in the quality control laboratory.

References

[1] A.G. Gilman and L.S. Goodman, in *The Pharmacological Basis of Therapeutics*, 7th edn, pp. 202–203. Macmillan, New York (1985).
[2] British Pharmacopoeia, Vol. II, 903 (1988).
[3] British Pharmacopoeia, Vol. I, 49 (1988).
[4] L.R. Snyder, *J. Chromatogr. Sci.* **16**, 223–234 (1978).
[5] L.R. Snyder, *J. Chromatogr.* **92**, 223–230 (1974).
[6] P.J. Schoenmakers, H.A.H. Billet and L. De Galan, *J. Chromatogr.* **205**, 13–30 (1981).
[7] L.R. Snyder, J.W. Dolan and J.R. Gant, *J. Chromatogr.* **165**, 3–8 (1973).
[8] J.C. Berridge (Ed.), *Techniques for the Automated Optimisation of HPLC Separations*, pp. 125–135. Wiley (1986).
[9] A.G. Wright, A.F. Fell and J.C. Berridge, *Chromatographia* **24**, 533–541 (1987).

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