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# Short Communication

# A high-performance liquid chromatographic method for the monitoring and quantification of the synthesis of *p*-hydroxyphenylacetamide

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ABSTRACT

*p*-Hydroxyphenylacetamide (HPAD) is the precursor for the synthesis of the β-blocker atenolol. A high-performance liquid method using methanol–water–tetrahydrofuran–acetic acid (35:65:1:1, v/v) as the mobile phase, a Novapak-$C_{18}$ column and detection at 254 nm was developed for the monitoring and quantification of the synthesis of HPAD. The method allows the simultaneous determination of the product, the starting material and the intermediates and thereby permits the optimization of the reaction parameters to obtain impurity-free HPAD.

## INTRODUCTION

Atenolol is a potent β-blocker commonly prepared by the reaction of epichlorohydrin with *p*-hydroxyphenylacetamide (HPAD) and its subsequent condensation with isopropylamine [1,2]. There are several routes for the synthesis of HPAD [3–5], but in this study a four-step synthesis starting from *p*-hydroxyacetophenone (PHA) was adopted [6] (Fig. 1).

After optimization of the high-performance liquid chromatographic (HPLC) conditions, the product was found to give rise to four well resolved peaks, which were identified and subsequently quantified. These results permitted the optimization of the synthesis parameters to obtain pure samples of HPAD.

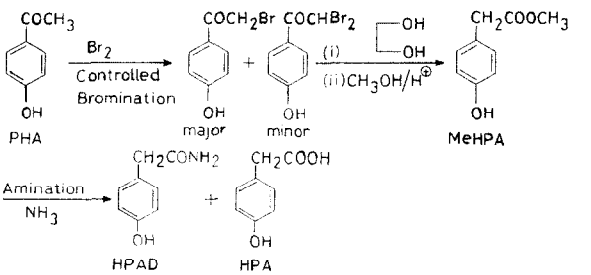


Fig. 1. Reaction scheme for the synthesis of HPAD from PHA.

## EXPERIMENTAL

### *Reagents*

The following reagent were used: *p*-hydroxyphenylacetic acid (HPA) (Sigma, St. Louis, MO, USA), *p*-hydroxyacetophenone (PHA) (Boehringer, Ingelheim, Germany), *p*-nitroacetophenone [internal

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standard (I.S.)] (Riedel-de Haën, Hannover, Germany), methyl ester of HPA (MeHPA) (synthesized from HPA in our laboratory) and hydroxyphenylacetamide (HPAD) (ICI, Mereside, UK).

*Instrumentation*

The HPLC system consisted of a Model 6000 alternating pump, a Novapak-$C_{18}$ column (4-$\mu$m particle size) (150 mm × 3.9 mm I.D.), a Model 481 UV detector (all from Waters Assoc., Milford, MA, USA), a Rheodyne injector with a 10-$\mu$l loop and a Hewlett-Packard Model 3394A integrator.

*Chromatographic analyses*

The mobile phase was methanol–water–tetrahydrofuran–acetic acid (35:65:1:1, v/v) at a flow-rate of 1 ml/min. The detection wavelength was 254 nm and the sample size was 10 $\mu$l throughout.

Nine standard calibration mixtures with different concentrations of HPA, PHA, MeHPA and HPAD with *p*-nitroacetophenone as the internal standard were prepared and analysed by HPLC. Calibrations graphs were constructed for each analyte and the response factors determined from the slope. Synthetic mixtures of all four analytes were then prepared and analysed using the same system and the validity of the response factors was examined by checking the mass balance. The analysis of reaction mixtures and the final assay of pure HPAD were done using the same system.

## RESULTS AND DISCUSSION

The results for HPAD, HPA, PHA and MeHPA are given in Table I. It is evident that the response factor of PHA is significantly higher than those of the other analytes, being about 20 and 17 times those of MeHPA and HPA, respectively. It was therefore essential to take the response factors into account when determining the concentration of each constituent of the mixture.

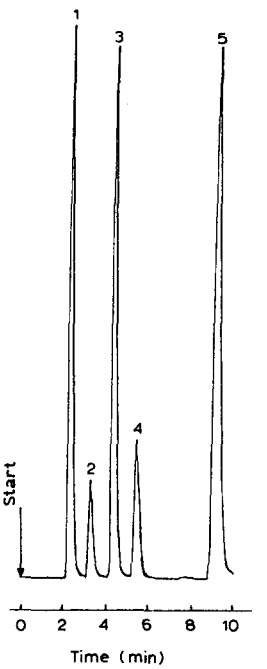


Fig. 2. Typical chromatogram of a synthetic mixture of analytes with an internal standard: (1) HPAD (4.94 $\mu$g); (2) HPA (0.86 $\mu$g); (3) PHA (0.29 $\mu$g); (4) MeHPA (1.61 $\mu$g); (5) I.S. (0.29 $\mu$g).

A typical chromatogram of a synthetic mixture of the analytes is shown in Fig. 2; all the peaks are baseline resolved. The calibration for each analyte

TABLE I

RELATIVE RETENTION TIMES (*RRT*), RESPONSE FACTORS (*RF*), CAPACITY FACTORS, RESOLUTION ($R_s$) AND LINEARITY RANGE OF THE CONSTITUENTS OF THE HPAD SYNTHESIS ROUTE

| Compound | *RRT* | *RF* | Capacity factor | $R_s$ | Linearity range ($\mu$g) |
|---|---|---|---|---|---|
| HPAD | 0.22 | 0.039 | 0.41 | – | 0.32 – 5.13 |
| HPA | 0.33 | 0.044 | 1.14 | 2.45 | 0.33 – 3.00 |
| PHA | 0.44 | 0.762 | 1.90 | 2.55 | 0.71 – 1.54 |
| MeHPA | 0.58 | 0.038 | 2.80 | 2.18 | 0.32 – 2.73 |
| I.S. | 1 | 1 | 5.52 | 5.63 | – |

TABLE II

LINEARITY OF RESPONSE FOR THE DIFFERENT ANALYTES

| Compound[a] | Sample No. | Area / Area I.S. | Conc. / Conc. I.S. | Area ratio / Conc. ratio | Compound[a] | Sample No. | Area / Area I.S. | Conc. / Conc. I.S. | Area ratio / Conc. ratio |
|---|---|---|---|---|---|---|---|---|---|
| HPA | 1 | 0.053 | 1.17 | 0.045 | MeHPA | 1 | 0.042 | 1.13 | 0.037 |
| | 2 | 0.109 | 2.34 | 0.046 | | 2 | 0.085 | 2.25 | 0.021 |
| | 3 | 0.157 | 3.51 | 0.045 | | 3 | 0.128 | 3.38 | 0.038 |
| | 4 | 0.210 | 4.68 | 0.045 | | 4 | 0.183 | 4.50 | 0.040 |
| | 5 | 0.254 | 5.85 | 0.043 | | 5 | 0.217 | 5.63 | 0.039 |
| | 6 | 0.312 | 7.02 | 0.044 | | 6 | 0.261 | 6.76 | 0.039 |
| | 7 | 0.356 | 8.19 | 0.043 | | 7 | 0.303 | 7.88 | 0.038 |
| | 8 | 0.405 | 9.36 | 0.043 | | 8 | 0.348 | 9.01 | 0.039 |
| | 9 | 0.463 | 10.53 | 0.044 | | 9 | 0.394 | 9.57 | 0.041 |
| HPAD | 1 | 0.072 | 1.12 | 0.064 | PHA | 1 | 0.545 | 0.60 | 0.905 |
| | 2 | 0.103 | 2.25 | 0.046 | | 2 | 0.951 | 1.21 | 0.792 |
| | 3 | 0.193 | 4.49 | 0.043 | | 3 | 1.412 | 1.81 | 0.779 |
| | 4 | 0.283 | 6.75 | 0.042 | | 4 | 1.904 | 2.41 | 0.788 |
| | 5 | 0.348 | 8.99 | 0.039 | | 5 | 2.321 | 3.01 | 0.771 |
| | 6 | 0.450 | 11.24 | 0.040 | | 6 | 2.868 | 3.62 | 0.792 |
| | 7 | 0.523 | 13.49 | 0.039 | | 7 | 3.164 | 4.22 | 0.749 |
| | 8 | 0.060 | 15.74 | 0.038 | | 8 | 3.640 | 4.75 | 0.766 |
| | 9 | 0.706 | 17.99 | 0.039 | | 9 | 4.131 | 4.43 | 0.761 |

[a] Regression equations:

$y = (5.181 \cdot 10^{-3}) + (4.319 \cdot 10^{-2})C_{HPA}$

$y = (2.421 \cdot 10^{-2}) + (3.738 \cdot 10^{-2})C_{HPAD}$

$y = (-5.508 \cdot 10^{-3}) + (4.011 \cdot 10^{-2})C_{MeHPA}$

$y = (7.854 \cdot 10^{-3}) + (7.476 \cdot 10^{-1})C_{PHA}$.

was done for the concentration ranges given in Table I and the response was found to be linear for all four analytes (Table II). The inter-assay precision of the method was established by triplicate analyses of synthetic mixtures of different compositions and the percentage error was found to be generally less than 1% with a maximum error of 1.09% (Table III).

The results of the analysis of actual reaction mixtures is shown in Table IV, where the difference between the percentage concentration of each analyte determined from the zero method (area percentage) and that obtained after taking the relevant response factors into account is clearly evident. The results of the analysis of reaction mixtures were in good agreement with the yields obtained after isolating

TABLE III

RESULTS OF THE ANALYSIS OF SYNTHETIC MIXTURES OF THE ANALYTES

Averages of triplicate determinations.

| HPAD (%) | | | HPA (%) | | | MeHPA (%) | | | PHA (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Taken | Found | Error | Taken | Found | Error | Taken | Found | Error | Taken | Found | Error |
| 63.90 | 63.78 | 0.12 | 11.50 | 11.13 | 0.37 | 20.84 | 20.78 | 0.06 | 3.76 | 3.77 | 0.01 |
| 65.62 | 65.13 | 0.49 | 8.33 | 8.33 | 0.00 | 17.90 | 16.81 | 1.09 | 8.18 | 7.62 | 0.56 |
| 59.19 | 58.87 | 0.32 | 21.31 | 21.50 | 0.19 | 8.48 | 8.43 | 0.05 | 11.00 | 10.22 | 0.78 |

TABLE IV

RESULTS OF THE ANALYSIS OF REACTION MIXTURES FOR THE SYNTHESIS OF HPAD

| Sample | Analyte | Area (%) | Actual [a] (%) |
|---|---|---|---|
| I | HPAD | 78.00 | 87.00 |
| | HPA | 4.50 | 4.78 |
| | PHA | 17.22 | 1.00 |
| | MeHPA | – | – |
| II | HPAD | 90.40 | 90.95 |
| | HPA | 3.50 | 3.13 |
| | PHA | 6.00 | 0.32 |
| | MeHPA | – | – |

[a] After correction with respective response factors.

the product. The presence of HPA in the reaction mixture (Table IV) suggested the possible hydrolysis of MeHPA to HPA and the synthesis parameters were accordingly optimized to minimise this hydrolysis.

The method described is effective for monitoring the synthesis and for the final assay of the product. With minor modification it should also be possible to use this method for monitoring the synthesis of HPAD by other routes. In fact, a similar system has been used for monitoring the synthesis of HPAD starting from sodium hydroxymandelate and involving HPA as an intermediate [7].

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