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Short Communication

High-performance liquid chromatographic separation of intermediate products and potential impurities by the synthesis of roxatidin

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ABSTRACT

A high-performance liquid chromatographic method is described for routine analytical control of the synthesis of roxatidin —a new β-blocker. Under control are three groups of compounds: starting products, intermediates and final substance. A reversed-phase RP-18 column for the first and last groups and an RP-2 column for the intermediates are preferred, although all three stationary phases are effective. The mobile phases are methanol–water (30:7, v/v) and tetrahydrofuran–acetonitrile–buffer (20:10:70, v/v/v) for the RP-18 column and tetrahydrofuran–acetonitrile–water (25:5:70, v/v/v) for the RP-2 column. The intra-assay precision is about 2% for the principal component in the chromatograms.

INTRODUCTION

Roxatidin, N-{3-[3-(1-piperidylmethyl)phenoxy]propyl}-acetoxyacetamide hydrochloride, is a new H_2 blocker with a marked effect on benign and post-operative ulcer or duodendal ulcer. Its structure is different from the other known β-blockers and its synthesis requires the analysis of a series of compounds as given in Table I.

To the best of our knowledge only one analytical method for the analysis of roxatidin, a thin-layer chromatographic method, exists in the literature [1]. Thus we are faced with the problem of analysing the compounds listed. They have a low vapour pressure and some of them are amides, *e.g.* with a low thermal stability. All of the compounds absorb in UV light. Therefore a liquid chromatographic method with UV detection seems to be the most suitable analytical method. According to the technological scheme a smaller subset of these compounds needs to be separated in one run. Based on our experience in routine analysis in factory laboratories, we prefer isocratic elution and reversed-phase columns.

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EXPERIMENTAL

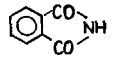
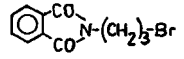
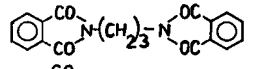
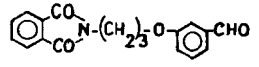
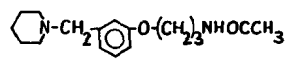
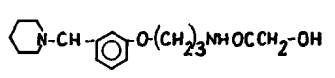
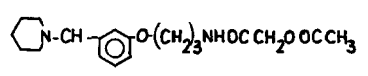
Equipment

A Pye Unicam LXD pump equipped with a Model 4025 UV detector and LiChrosorb RP-18, RP-8 and RP-2 columns, 250 × 4.6 mm I.D. and 10 μm particle size, from Merck (Darmstadt, Germany) were used. The integrator was a Waters (Milli-

0021-9673/92/$05.00

TABLE I

STRUCTURES OF COMPOUNDS

No.	Structure	Abbreviation	Compound name
1		PhI	Phthalimide
2		BPrPh	Brompropylphthalimide
3		DPhPr	1,3-Diphthalimidopropane
4		PhPrBA	3-Phthalimidopropoxybenzaldehyde
5		AA	N-{3-[3-(1-Piperidinylmethyl)phenoxy]propyl}acetamide
6		HAA	N-{3-[3-(1-Piperidinylmethyl)phenoxy]propyl}hydroxyacetamide
7		Rx	N-{3-[3-(1-Piperidinylmethyl)phenoxy]propyl}acetoxyacetamide hydrochloride (roxatidin)

pore, Milford, MA, USA) Model 740. The appropriate analytical wavelength is 278 ± 2 nm.

Chemicals

The organic modifiers tetrahydrofuran (THF), acetonitrile and methanol were from Merck and Fluka (Buchs, Switzerland) and were HPLC grade. *o*-Phthalimide (PhI) and *m*-hydroxybenzaldehyde (BA) were from Fluka. Roxatidin was obtained in our laboratory and its properties were compared with roxatidin extracted from existing market formulations. All other compounds were synthesized in NIHFI. After checking the purity, their structure was verified by IR and ^{1}H NMR.

Mobile phases

According to the structure of compounds, (Table I), two different types of mobile phases were tested: "neutral" and "buffered". The buffer consisted of 0.1 *M* orthophosphoric acid, the pH of which was adjusted to 3.0 with triethylamine (TEA). A flow-rate of 1.0 ml/min was set in all cases. The exact compositions are given in the legends to the figures.

RESULTS AND DISCUSSION

Starting products

m-Hydroxybenzaldehyde, although "acidic", emerged as a symmetrical peak from an RP-18 column with methanol–water (30:70, v/v) and was well separated from *o*-phthalimide. Changing the column to RP-8 or RP-2 necessitated only a small change in the mobile phase composition.

Intermediates

The target product from the first stage of the synthesis was brompropylphthalimide (BPrPh). The sample from the second stage consisted of one desired compound, 3-phthalimidopropoxybenzaldehyde (PhPrBA), and as impurities BPrPh and by-products, the majority of which was diphthalimidopropane (DPhPr).

Quantitation of these impurities necessitated a baseline separation. The sequence of the peaks, using acetonitrile as modifier, was DPhPr, BPrPh, PhPrBA, and the resolution (R_s) from the target compound was 1.4; however, neither DPhPr nor BPrPh could be quantified correctly, because of overlapping (R_s < 0.8). The sequence of the peaks

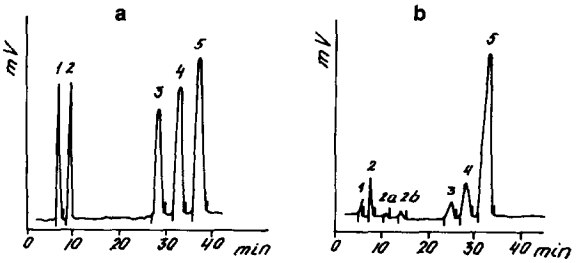


Fig. 1. (a) HPLC profile of a mixture of reference substances (total concentration 2 mg/ml) eluted onto a LiChrosorb RP-2 column. Detection at 280 nm. Mobile phase: tetrahydrofuran–acetonitrile–water (27.0:4.0:69.0, v/v/v). Peaks: 1 = PhI; 2 = BA; 3 = BPrPh; 4 = DPhPr; 5 = PhPrBA. For abbreviations, see Table I. (b) As in (a) but with a real sample of PhPrBA (about 1 mg/ml). Peaks 2a and 2b are unknown.

of DPhPr and BPrPh was reversed when tetrahydrofuran was used as the mobile phase and on RP-2 only their separation could be improved by reducing the content of tetrahydrofuran. The analysis time, however, increased to more than 40 min. An iterative method described in ref. 2 was applied for further optimization. The analysis time (the retention time of the last peak) was accepted to be not more than 30 min and two isoelutropic mobile phases were prepared to meet this demand. Following the optimization steps as given in ref. 2, the mixture tetrahydrofuran–acetonitrile–water (25.0:5.0:70.0, v/v/v) was accepted as adequate for the necessary separation (Fig. 1). All components were resolved to the baseline. The relative retention (RR), given as k'_i/k'_{BPrPh}, and the separation presented by R_s are given in Table II.

TABLE II

RELATIVE RETENTION (RR) AND R_S VALUES ON AN RP-2 COLUMN FOR THE COMPOUNDS UNDER INVESTIGATION

No.	Compound	RR	R_s
1	PhI	0.13	1.1
2	*m*-Hydroxybenzaldehyde	0.16	>2
3	DPhPr	0.85	1.2
4	BPrPh	1.00	1.2
5	PhPrBA	1.15	

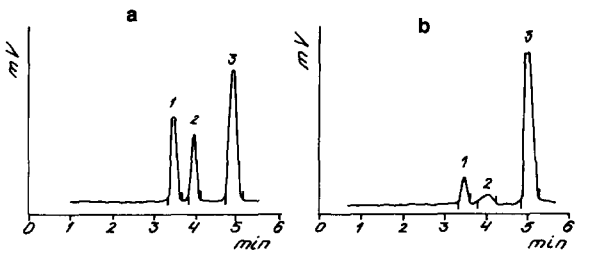


Fig. 2. HPLC profile of a mixture of roxatidin base and HAA eluted onto a LiChrosorb RP-18 column (about 0.3 mg/ml each). Detection at 280 nm. Mobile phase: methanol–acetonitrile–buffer (30:10:60, v/v/v). Buffer: 0.1 *M* phosphoric acid adjusted to pH 3.0 with TEA. Peaks: 1 = HAA; 2 = AA (see text); 3 = Rx. For abbreviations, see Table I. (b) As in (a) but with a real sample of roxatidin (1 mg/ml).

Substance

Roxatidin could be accompanied by the preceding product in its synthesis: N-{3-[3-(1-piperidinylmethyl)phenoxy]propyl}hydroxyacetamide (HAA) (Table I). With an RP-18 column and methanol–acetonitrile–buffer (20:10:70, v/v/v) as the mobile phase a baseline separation was achieved. The impurities emerged before the base peak (Fig. 2). The problem which arose in this stage of the synthesis was the second peak. Neither of the compounds in the Table I gave this peak. It was assumed that it belongs to the compound N-{3-[3-(1-piperidinylmethyl)phenoxy]propyl}acetamide (AA) (see Table I). By synthesizing this compound and employing chromatography under various conditions it was confirmed that the second peak is due to this compound.

Method validation

The amount of tetrahydrofuran is crucial to the resolution of DPhPr and PhPrB, which is why its optimum content should be determined experimentally for every column, after good conditioning. Solutions of BPrPh are unstable and they must be freshly prepared before analysis (storage time not longer than 20 min.)

The linearity of the detector response against roxatidin was verified from 0.05 to 0.5 mg/l. The limit of detection at a signal-to-noise ratio of 5 for AA and HAA was 2 μg/ml.

The intra-assay precision (eight injections) was about 2% for the principal component in the chromatogram and increased to *ca.* 3–4% for components present in a concentration of about 1%. The inter-assay precision (between first and third and third and fifth days) was 2.2% and 5%, respectively.

If the purity of PhPrBA is the problem, the concentration of the sample solution in the mobile phase must be 1 mg/ml. In this case, because of the non-linearity of the response, quantitation of the impurities was made by the external standard method using a dilute solution of PhPrBA.

Whichever approach was used, the principal compound of every stage of the synthesis could be determined accurately: in the first stage the separation of PhI and BA could be performed onto an RP-18 column with methanol–water as the mobile phase; in the next stage, again with an RP-18 column, both DPhPr and BPrPH were eluted together but separately from PhPrBA. We have endeavoured to use a limited range of conditions using an RP-2 column. This approach worked very well with the compounds from this stage that are difficult to chromatograph, and eluted the principal component at last. At the last stage the purity of roxatidin was also determined very well using again an RP-18 column with a ternary mobile phase. Thus, the proposed chromatographic methods allow the qualitative and quantitative control of the whole process of roxatidin synthesis.

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