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A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high-performance liquid chromatography

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Abstract: This paper describes a general approach for the therapeutic drug monitoring of 13 different beta blockers in plasma. The chromatographic system contains a cyanopropyl-bonded phase as a stationary phase in combination with a mobile phase composed of acetonitrile and phosphate buffer (pH = 3, μ = 0.05). Two modes of detection are used, namely, UV detection and fluorescence detection. The sample pretreatment is performed with a nitrile-sorbent in combination with methanol–phosphate buffer (pH = 3, μ = 0.05) or with methanol containing 0.1% propylamine as eluent. Acceptable recoveries are obtained for practolol, acebutolol, pindolol, oxprenolol, mepindolol, atenolol, propranolol, prenalterol, alprenolol, metoprolol, sotalol and nadolol. For labetalol, however, the elution recovery has to be improved. Finally, this approach is illustrated by the assay of nadolol in the plasma of patients suffering from hypertension, who had received an oral formulation of the drug.

Keywords: *Beta blockers; therapeutic drug monitoring in plasma; solid-phase extraction off-line; liquid chromatography.*

Introduction

The measurement of beta adrenoceptor antagonists in plasma, offers useful information for clinical studies of new beta blockers [1] in cases of intoxication, in controlling the therapy compliance of the patients, in the study of possible pharmacokinetic interactions with other drugs (lipid soluble beta blockers with calcium antagonists, for instance) and also in the doping control, since these drugs have recently been abused in sports such as snooker, shooting, ski jumping etc., and are included on the list of drugs banned by the International Olympic Committee [2].

Furthermore, it is known that there is wide variability in plasma concentrations observed inter- as well as intra-individually for this group of drugs, owing to their irregular absorption and extensive first-pass metabolism [3, 4].

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The literature offers many articles describing the determination of a particular beta blocker and its possible metabolites in plasma [4–23]. These include a large variety of analytical methods and associated sample pretreatment procedures.

Lefebvre *et al.* [9] developed a method to find the best conditions for extraction and detection of nine different beta-blocking agents. The type of sample pretreatment used was liquid–liquid extraction. Winkler *et al.* [10] developed an HPLC method for the quantitative analysis of three beta blockers: propranolol with a large log *P* value, metoprolol with an intermediate polarity and atenolol possessing a very low log *P* value. They assumed that the method could be applied to the whole group of beta blockers using the three beta blockers mentioned as representative compounds. These authors also used liquid–liquid extraction.

The purpose of this paper is to describe the development of a general strategy that allows the assay of 13 different beta blockers in plasma, in the therapeutical range. The analytical technique selected was HPLC with off-line solid-phase extraction (SPE) as the sample preparation step. To the best of the authors' knowledge such a general approach using SPE has not previously been described in the literature. The approach has the advantage that the sample pretreatment is less time consuming than with liquid–liquid extraction, and more compatible with automatization.

In earlier work [24] it was stated that the use of a cyanopropyl bonded phase in combination with a mobile phase containing acetonitrile and phosphate buffer (pH = 3, μ = 0.05), offers in general acceptable conditions for the chromatography of basic compounds. These parameters are compatible with the three common detection systems used in HPLC, namely UV, fluorescence and amperometric detection. In this study the use of such chromatographic systems was investigated for the determination of beta blockers in plasma.

A general approach for the isolation of basic drugs from plasma using a CN-sorbent in combination with methanol–phosphate buffer or methanol containing 0.1% propylamine as eluent, has been described elsewhere [25]. For the validation of this methodology, beta adrenoceptor antagonists form a suitable group since they can be considered as relatively strong bases; the mean value of the acid dissociation constant is reported as 9.4 [6] and on the other hand, there is a great variety in the lipophilicity within this group of compounds [6] which makes the validation of the extraction procedure described [25] more attractive. Besides, one can hope that from this investigation, rules can be derived based on the characteristics of the drug, providing initial conditions for the assay of a new beta blocker in plasma.

Finally this approach was applied for the assay of nadolol in plasma of patients suffering from hypertension. These assays were required for a comparative study of a new non-cardioselective beta blocker, tertatolol [26, 27].

Experimental

Apparatus

The chromatograph used consisted of a Model 5000 pump (Varian, Walnut Creek, USA), a Rheodyne injector (injection volume = 100 μl) and a fluorescence detector LS 4 (Perkin–Elmer, Ueberlingen/See, FRG). The detector sensitivity was set at its highest value (4) and the slits were at setting (10) both for excitation and emission. Chromatograms were monitored and integrated with a Vista CDS — 401 instrument (supplied by Varian).

A second instrument consisting of a Perkin–Elmer pump series 10 coupled in series with a UV detector with variable wavelength (optical pathlength = 1 cm) LC 90 UV also from Perkin–Elmer. As integrator, an IC-R3A was used (Shimadzu, Kyoto, Japan). The vacuum manifold device SPE 21 and the CN cartridges (1 ml capacity) were provided by J. T. Baker (Deventer, The Netherlands).

LiChrosorb cyanopropyl bonded (12.5 × 0.4 cm, i.d.; particle size, 5 μm) columns were used as supplied by Merck (Darmstadt, FRG). The analytical column was protected by a guard column containing LiChrosorb CN-bonded 10 μm silica.

Chemicals

The reagents were purchased from Merck (Darmstadt, FRG). Acetonitrile was of liquid-chromatographic grade; methanol of analytical grade. Phosphoric acid and sodium dihydrogen phosphate were also obtained from Merck. The water used in all experiments was purified on a Milli-Q system (Millipore, Molshein, France). Before use, the buffer was filtered through a 0.2-μm membrane filter. Propylamine was obtained from Fluka (Buchs, Switzerland). Surfasil (Pierce, Oud Beyerland, The Netherlands), diluted 10 times with acetone, was used to silanize all the glass items used in the experiments. The stock solutions of the beta blockers were prepared by dissolving 100 ppm of these compounds in methanol. They were stored at 4°C. They were further diluted with water for loading the plasma samples and with methanol–phosphate buffer or with the mobile phase to measure the recoveries. These diluted solutions were prepared fresh each day.

Sample pretreatment

Blank human plasma and patient samples containing nadolol were stored at −20°C. They were allowed to thaw and warm up to room temperature. From this pool of blank plasma, aliquots of 1 ml were transferred to a silanized glass tube and spiked with 100 μl of an aqueous drug standard. The deproteinization was performed by adding, dropwise and under vortexing, 2 ml of acetonitrile. After centrifugation, the supernatant was transferred to a cartridge conditioned with 2 ml of methanol followed by 2 ml of water. The cartridge was then rinsed with 3 ml of water and the drug was recovered using 1 ml of eluent [methanol–phosphate buffer, pH = 3, μ = 0.05, (50:50, v/v); or methanol +0.1% propylamine). The former eluent was injected directly into the chromatographic system, whilst the latter was evaporated and then reconstituted in 1 ml of the mobile phase.

Results and Discussion

Chromatographic conditions

Selection of adequate stationary and mobile phases. The use of a cyanopropyl-bonded phase as the stationary phase in combination with a mobile phase composed of acetonitrile and phosphate buffer (pH = 3, μ = 0.05), provides valid conditions for the chromatography of a wide range of drugs [24]. Furthermore, these chromatographic conditions are compatible with UV, amperometric (oxidative mode) and fluorescence detection so that the analyst is able to enhance the sensitivity and/or the selectivity in cases where those parameters are not sufficiently high enough using UV detection alone.

For each beta blocker, the percentage of organic modifier (acetonitrile) present in the mobile phase was adjusted so that acceptable k' values were obtained. This means that

the ideal k' value is a compromise; it should be high enough so that the compound is sufficiently separated from possible plasma interferences, whilst on the other hand, it should not be too high, e.g. ~5.0, in order to minimize analysis times. The results are presented in Fig. 1. On the basis of which three chromatographic systems were selected as follows: (i) for labetalol, propranolol and mepindolol, 20% of acetonitrile; (ii) for acebutolol, alprenolol, metoprolol, oxprenolol and pindolol, 10% of organic modifier;

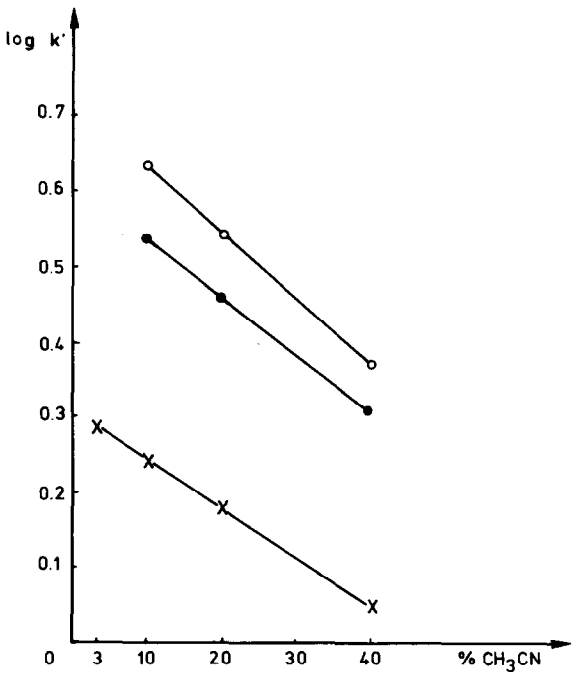

Figure 1
Log k' as a function of the amount of acetonitrile present in the mobile phase: ○, propranolol; ●, labetalol; ×, nadolol.

Table 1
Chromatographic conditions and lipophilicity properties of 13 beta-blocker drugs

Compound	% Acetonitrile in eluent	Capacity ratio, k'	Number of carbon atoms	log P
Mepindolol	20	1.86	15	—
Propranolol	20	3.47	16	3.65
Labetalol	20	2.88	19	3.18
Pindolol	10	2.57	14	1.75
Alprenolol	10	3.47	15	2.61
Metoprolol	10	1.86	15	2.15
Oxprenolol	10	2.69	15	2.18
Acebutolol	10	2.14	18	1.87
Sotalol	3	1.20	12	−0.79
Prenalterol	3	1.23	12	—
Atenolol	3	1.45	14	0.23
Practolol	3	1.70	14	0.79
Nadolol	3	1.95	17	0.71

(iii) for atenolol, nadolol, prenalterol, sotalol and practolol, 3% of acetonitrile. The degree of polarity expressed as log P value is correlated with the capacity factor, k' (see Table 1).

Selection of a suitable detection system. Generally, optical detection systems, such as UV and fluorescence, are preferred to amperometric detection because they are more user friendly. Furthermore, it is known that the sensitivity obtained with fluorescence detection is the same or even better than that provided by amperometric detection. Besides, fluorescence detection also offers better selectivity. In the present work, UV detection at the analyte absorption maximum was used, to be replaced by fluorescence detection when the therapeutical concentrations could not be monitored by UV detection. Eleven of the compounds of interest show intrinsic fluorescence activity, the exceptions being practolol and oxprenolol. For each compound dissolved in the mobile phase, the excitation and emission wavelengths were determined; the results being summarized in Table 2.

For UV detection the absorption maximum wavelength was used, as shown in Table 2. Alternatively, 220 nm was selected for those compounds where the molar absorptivity at 220 nm was higher than that at the absorption maximum, e.g. pindolol and oxprenolol. The therapeutical concentrations of the remaining seven beta blockers were determined with fluorescence detection.

Extraction procedure

In an earlier work, a general approach for the isolation of basic drugs from plasma was described [25]. This involved the use of a CN-sorbent, which was conditioned with two column volumes of methanol and 2 ml of water. These phases totally retain basic drugs possessing a carbon chain ≥ 11 and it is possible to rinse the cartridge with 3 × 1 ml of water without loss of the analyte. Methanol–phosphate buffer (pH = 3, μ = 0.05, 50:50, v/v) and methanol containing 0.1% propylamine provide acceptable elution recoveries.

First, this approach was tried for the isolation of 13 beta blockers from water. As expected, all compounds were totally adsorbed from water on the CN-sorbent. The recommended rinsing procedure could also be applied without significant loss of the beta

Table 2
Ultraviolet absorption maxima and fluorescence excitation and emission wavelengths of 13 selected beta blockers

Compound	UV max	Fluorescence detection λ_{ex}	Fluorescence detection λ_{em}
Propranolol	220 ; 289	254	340*
Mepindolol	220 ; 267	255	315
Labetalol	220 ; 305	335	370
Pindolol	220 ; 265	255	315
Oxprenolol	220 ; 273	—	—
Metoprolol	224	230	305*
Alprenolol	220 ; 270	230	300*
Acebutolol	235	230	330*
Prenalterol	225	230	330*
Sotalol	232	280	305*
Atenolol	227	230	330*
Practolol	247	—	—
Nadolol	220 ; 279	230	300*

blocker of interest. Finally, the elution recoveries were measured to yield the data presented in Table 3.

Both eluents offer acceptable recoveries, except for labetalol. This can be explained by the fact that labetalol is a relatively apolar compound; it contains the highest number of carbon atoms of the test compounds, namely 19, and is characterized by a high log *P* value. The fact that methanol with 0.1% propylamine offers only 50% elution recovery, can be due to the relatively low pK_a value (7.4). It is known that elution can be difficult using a CN-sorbent for the isolation of relatively apolar neutral drugs from water such as estramustine and triamcinolone (unpublished results).

For the other more lipophilic beta blockers such as propranolol, with an even higher log *P* value than labetalol and acebutolol, containing 18 carbon atoms, methanol with propylamine yields acceptable recoveries, since the respective pK_a values are significantly higher (9.5).

For the other substances one can choose methanol–phosphate buffer (pH = 3, μ = 0.05, 50:50, v/v). This permits direct injection since the eluent is compatible with the mobile phases used. In those cases where other types of mobile phases are preferred that are incompatible with the above eluent, for instance normal phase conditions, or if trace enrichment is required, methanol containing 0.1% propylamine often provides analogous recoveries.

For this reason both eluents were also tried out for the extraction of beta blockers from plasma. In a first attempt, the extraction procedure was carried out at a level of 1 μg ml^{-1} plasma. A 1.0 ml vol of plasma was deproteinized by the addition of 2 ml of acetonitrile. After centrifugation the supernatant was evaporated and reconstituted in 1 ml of water and transferred to a conditioned cartridge. Water up to 3 ml was used as the rinsing solvent. The recoveries from plasma obtained for this set of beta blockers is presented in Table 4.

For six of the substances investigated, acceptable results were obtained; again an inferior recovery was obtained for labetalol. For sotalol, mepindolol, alprenolol, oxprenolol, metoprolol and pindolol the extracts contained no drug at all. The first possibility where a loss of the drug can occur, is the deproteinization step. Therefore, the extraction procedure was repeated for these six compounds in virtually the same way but

Table 3
Elution recoveries for the extraction of beta-blocker drugs from water

Compound	% Recovery with methanol–phosphate buffer (pH = 3, μ = 0.05, 1:1; *N* = 2)	% Recovery with methanol + 0.1% propylamine (*N* = 2)
Propranolol	90	91
Mepindolol	78	85
Labetalol	79	49
Pindolol	92	86
Alprenolol	90	101
Metoprolol	90	99
Oxprenolol	93	98
Acebutolol	96	83
Prenalterol	92	97
Sotalol	95	90
Atenolol	99	96
Practolol	86	98
Nadolol	92	86

Table 4
Recoveries for the extraction of beta blockers from plasma at the level of 1 μg ml^{-1} plasma

Compound	% Recovery with methanol–phosphate buffer (pH = 3, μ = 0.05; N = 2)	% Recovery with methanol + 0.1% propylamine (N = 2)
Propranolol	90	91
Mepindolol	0	0
Labetalol	71	68
Pindolol	0	0
Alprenolol	0	0
Metoprolol	0	0
Oxprenolol	0	0
Acebutolol	95	85
Prenalterol	72	88
Sotalol	0	0
Atenolol	80	92
Practolol	82	80
Nadolol	91	84

Table 5
Elution recoveries without deproteinization

Compound	% Recovery with methanol–phosphate buffer (pH = 3, μ = 0.05, 1:1; N = 2)	% Recovery with methanol + 0.1% propylamine (N = 2)
Sotalol	—	—
Mepindolol	69	66
Alprenolol	99	103
Oxprenolol	64	55
Metoprolol	97	85
Pindolol	96	82

without deproteinization. The recoveries were definitely better, except for sotalol. For alprenolol, metoprolol and pindolol, the extraction yields reached the acceptable values of 99, 97 and 96%, respectively, as shown in Table 5.

The loss in the deproteinization step could be the result of the coprecipitation of the drug or it could be explained by the fact that the reconstitution of the supernatant evaporated in 1 ml of water causes problems. In order to investigate the latter possibility, it was explored whether the hypernatant could be transferred directly onto the conditioned cartridge. First the retention, on a CN-sorbent, of these compounds from a medium containing 66%, v/v of acetonitrile was measured. All beta blockers investigated were totally retained. In this way the evaporation step is avoided and the total extraction procedure simplified. When this modified methodology was applied, the elution recoveries were significantly enhanced and even for alprenolol, metoprolol and pindolol the extraction yields were very good (see Table 5), indicating that the previously observed loss of the drugs was due to problems in the reconstitution step. However, for three of the compounds, this modification offered no significant improvement. Since it is known from earlier experiments that the competitive effect of the matrix on the adsorption of the drug is negligible [25], it can be concluded that the reduced recoveries were not due to the adsorption step. Accordingly, the possibility that matrix interferences could complicate the elution step was investigated. In the next experiment, the matrix was diluted with water in a ratio 1:5 so that the drug would not be so strongly

surrounded by plasma interferences and became more disposable for the eluent. Indeed, the dilution of plasma prior to the extraction procedure was found to improve the recoveries for oxprenolol, mepindolol and sotalol. For the first two beta blockers, this approach provides acceptable extraction yields, viz., 95% for oxprenolol and 94% for mepindolol. However, for sotalol the recovery was still unacceptable (Table 6).

Therefore, the matrix effect was reduced still further for sotalol. Instead of 1 ml of plasma, only 200 μl of plasma was used. This amount was diluted 1:5 with water. The extraction yield reached 80% when 1 ml of methanol–phosphate buffer (pH = 3, $\mu = 0.05$) was used as eluent. It should be noted that the dilution factor should not be too high, since larger amounts of eluent take more time to migrate through the sorbent and, furthermore, blockage of the upper frit of the cartridge can occur.

For each beta-blocker drug the extraction procedure described above was applied for the determination at therapeutical levels [3]. For the compounds for which the therapeutical plasma level was not found in the literature, the assays were performed at a level of 100 ng ml^{-1} plasma. The results are presented in Table 7.

Determination of nadolol in plasma samples of human volunteers

This assay was performed in connection with a randomized comparative study of nadolol versus tertatolol. The purpose of the determination of nadolol was to check whether the expected plasma concentrations (100–150 ng ml^{-1}) were reached, and secondly, to exclude randomization errors. The plasma samples analysed were from patients suffering from hypertension treated with a single dose of 80 mg nadolol *per os*.

Table 6
Elution recoveries with prior dilution of the plasma matrix

Compound	% Recovery with methanol–phosphate buffer ($N = 2$)	% Recovery with methanol + 0.1% propylamine ($N = 2$)
Oxprenolol	95	100
Mepindolol	94	86
Sotalol	61	47

Table 7
Extraction yields of 12 beta blockers determined at therapeutical plasma levels

Compound	Therapeutical plasma level (3)	% Recovery ($n = 6$)	Figure
Propranol	100 ng ml^{-1}	87.5 ± 7.2	2*
Mepindolol	100 ng ml^{-1}	94.4 ± 7.1	3
Pindolol	10 ng ml^{-1}	80.7 ± 7.3	4
Alprenolol	100 ng ml^{-1}	92.7 ± 7.6	5
Metoprolol	100 ng ml^{-1}	88.3 ± 5.7	6
Oxprenolol	100 ng ml^{-1}	85.0 ± 4.4	7*
Acebutolol	200 ng ml^{-1}	91.1 ± 5.5	8
Sotalol	1 μg ml^{-1}	84.6 ± 4.0	9
Prenalterol	100 ng ml^{-1}	92.9 ± 4.2	10*
Atenolol	100 ng ml^{-1}	85.4 ± 6.4	11*
Practolol	1 μg ml^{-1}	82.3 ± 3.9	12
Nadolol	100 ng ml^{-1}	91.1 ± 7.9	13
			14

*Eluent used in SPE procedure is methanol + 0.1% propylamine.

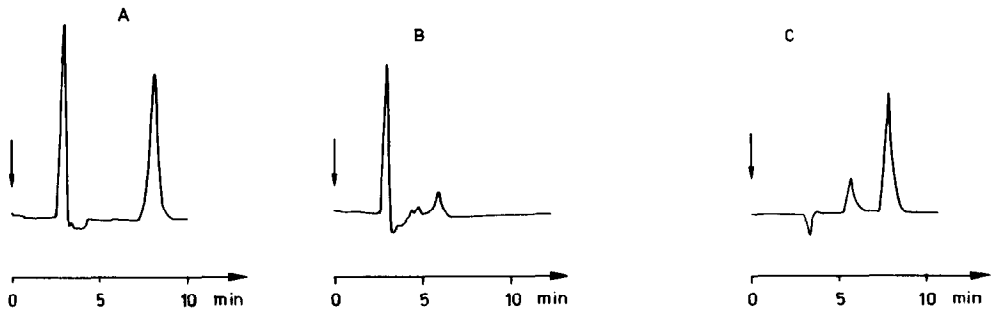

Figure 2
Chromatograms of practolol: A, standard of 1 μg ml^{-1} practolol in methanol–phosphate buffer; B, plasma blank; C, plasma extract of 1 ml plasma spiked with 1 μg practolol. The chromatograms were monitored at 247 nm and 0.02 a.u.f.s.

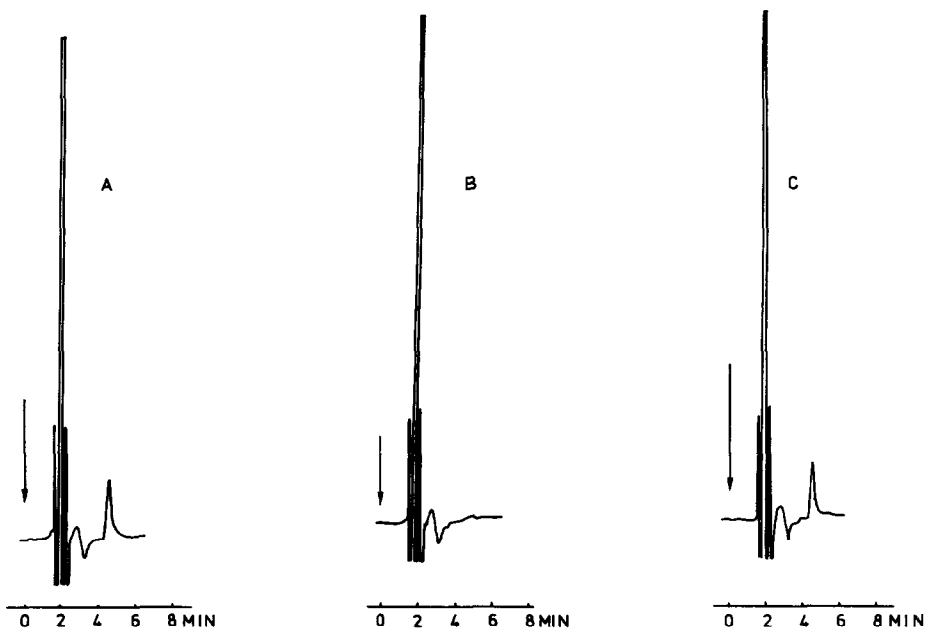

Figure 3
Chromatograms of acebutolol: A, standard of 200 ng ml^{-1} acebutolol in methanol–phosphate buffer; B, blank plasma; C, plasma extract of 1 ml plasma spiked with 200 ng acebutolol. UV detection at 235 nm was used at an attenuation of 0.005 a.u.f.s.

The recommended procedure was used as described, using methanol–phosphate buffer as eluent. The extract was injected directly into the HPLC system.

A calibration curve was set up by spiking 1 ml plasma vol with 0, 108, 216 and 324 ng nadolol. As internal standard, 500 ng viloxazine was added to each calibration point. The within day reproducibility was determined for the lowest level expected, namely 100 ng nadolol/ml plasma. The recovery obtained was 91.1% and the coefficient of

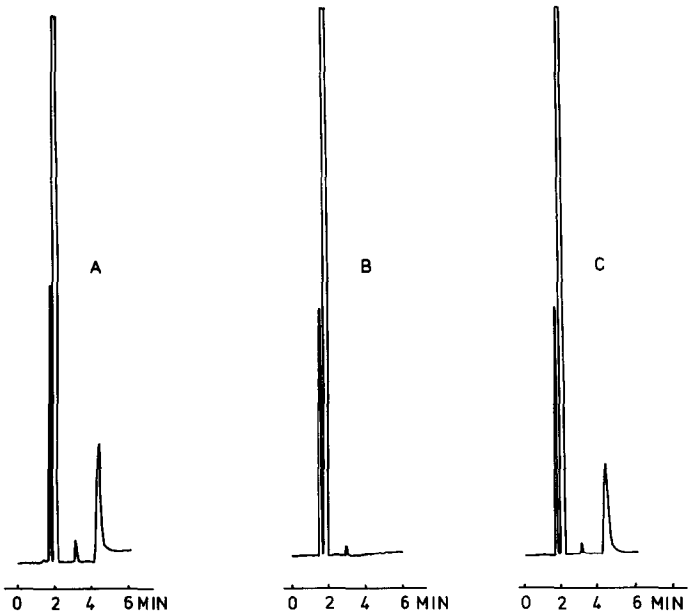

Figure 4
Chromatograms of pindolol: A, standard of 10 ng ml^{-1} in methanol–phosphate buffer; B, blank; C, plasma extract of 1 ml plasma loaded with 10 ng pindolol. UV detection at 220 nm and 0.005 a.u.f.s. was used.

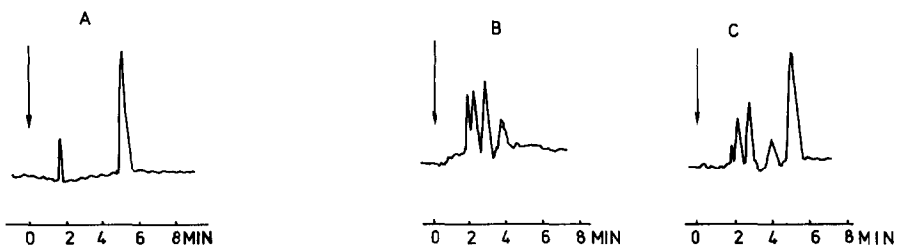

Figure 5
Chromatograms of oxprenolol: A, standard of 100 ppb oxprenolol dissolved in mobile phase; B, plasma blank; C, plasma extract of 1 ml plasma spiked with 100 ng oxprenolol. The chromatograms were monitored at 220 nm and 0.005 a.u.f.s.

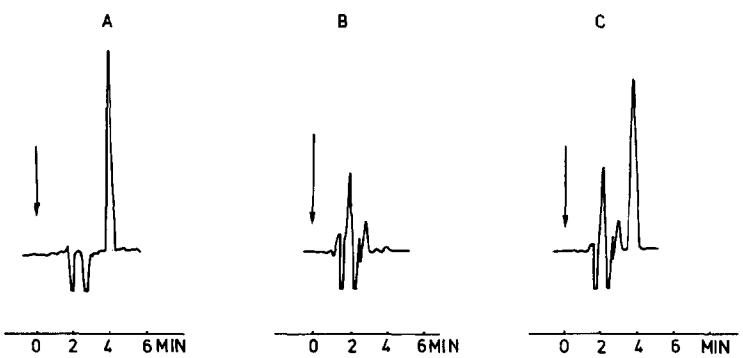

Figure 6
Chromatograms of mepindolol: A, standard of 100 ng mepindolol in methanol–phosphate buffer; B, plasma blank; C, plasma extract of 1 ml plasma loaded with 100 ng mepindolol. UV detection at 267 nm and 0.005 a.u.f.s. was used.

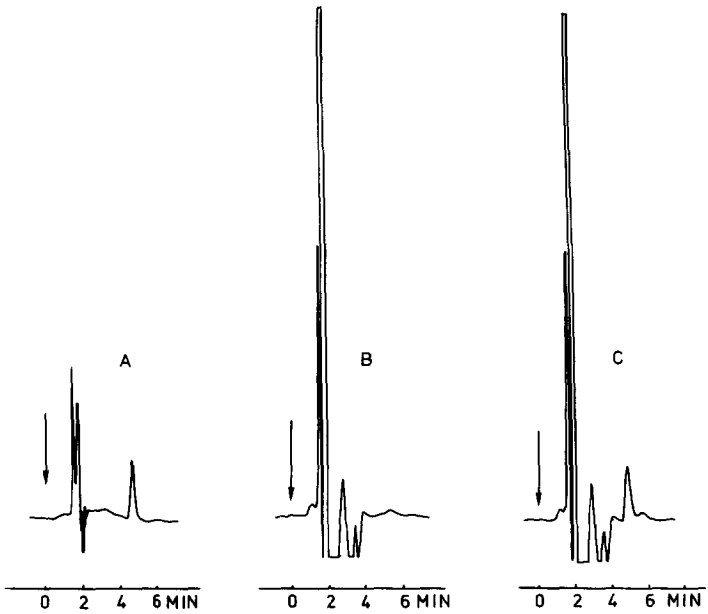

Figure 7
Chromatograms of atenolol: A, standard of 100 ng ml^{-1} atenolol in mobile phase; B, plasma blank; C, plasma extract of 1 ml plasma loaded with 100 ng atenolol. Fluorescence detection (230, 300) was used and the attenuation was set at a fixed scale of 1.

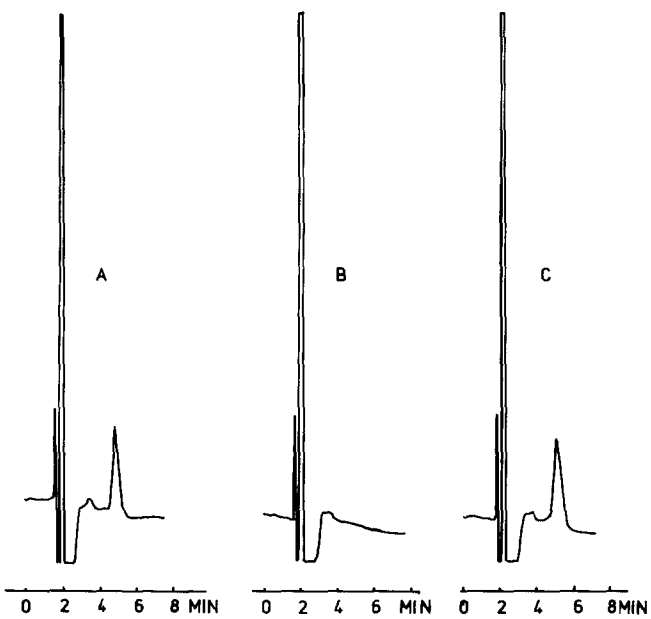

Figure 8
Chromatograms of propranolol: A, standard of 100 ng ml^{-1} propranolol dissolved in mobile phase; B, blank; C, plasma extract of 1 ml plasma loaded with 100 ng propranolol. The chromatograms were monitored at an excitation wavelength of 254 nm and an emission wavelength of 340 nm. The attenuation was set at a fixed scale of 1.

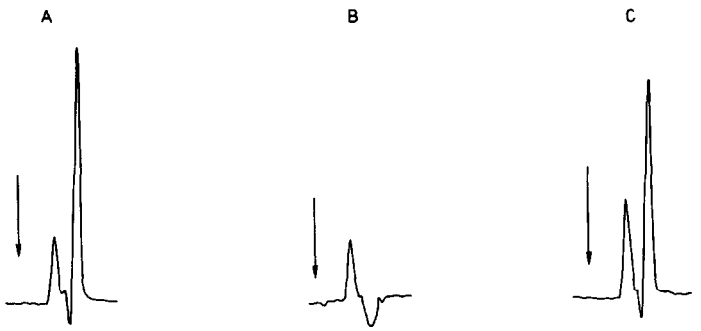

Figure 9
Chromatograms of prenalterol: A, standard of 100 ng ml^{-1} prenalterol in mobile phase; B, blank; C, plasma extract of 1 ml plasma spiked with 100 ng prenalterol. Fluorescence detection (230, 300) at a fixed scale of 1 was used.

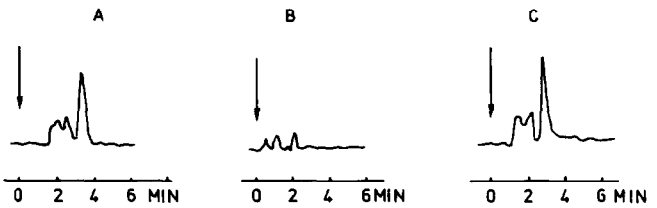

Figure 10
Chromatograms of alprenolol: A, standard of 100 ng alprenolol dissolved in methanol–phosphate buffer; B, plasma blank; C, plasma extract of 1 ml plasma spiked with 100 ng alprenolol. Fluorescence detection (230, 300) at a fixed scale of 1 was used.

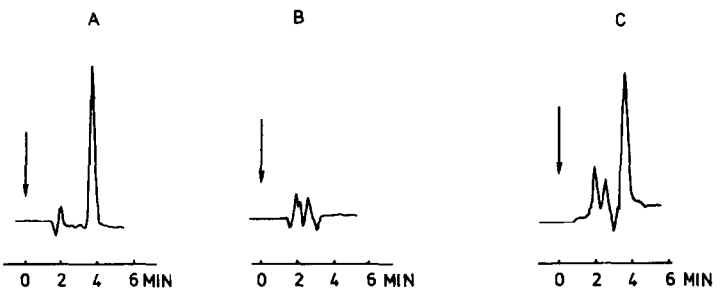

Figure 11
Chromatograms of metoprolol: A, standard of 100 ng metoprolol dissolved in methanol–phosphate buffer; B, plasma blank; C, plasma extract of 1 ml plasma loaded with 100 ng metoprolol. Fluorescence detection (230, 305) at a fixed scale of 1 was used.

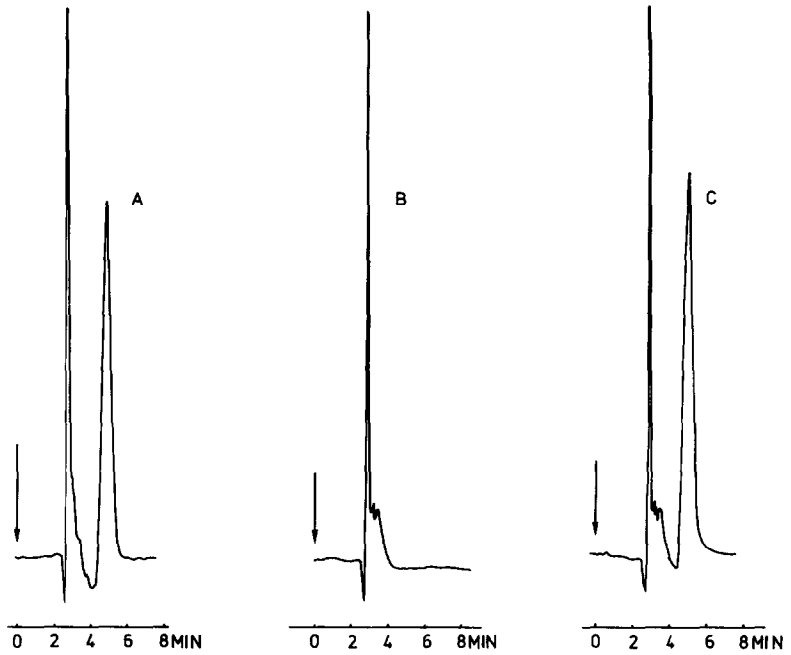

Figure 12
Chromatograms of sotalol: A, standard of 200 ng sotalol in methanol–phosphate buffer; B, plasma blank; C, plasma extract of 200 μl plasma loaded with 200 ng sotalol. Fluorescence detection (280, 305) at a fixed scale of 1 was used.

variation was 7.9% (n = 6). The chromatograms, including a plasma sample of a patient containing 81.6 ng nadolol/ml plasma are shown in Figs 13 and 14.

Conclusions

This work shows that it is possible to monitor therapeutical levels of beta blockers in plasma using a general approach. For the chromatographic system using a CN stationary phase, only the percentage acetonitrile had to be adjusted for each component of the set.

It was necessary to use two modes of detection (UV and fluorescence) since not all the beta blockers possess intrinsic fluorescence activity (viz., oxprenolol and practolol and, on the other hand, UV detection was not sensitive enough for the therapeutic drug monitoring of some beta blockers.

Concerning the sample pretreatment, it is possible to isolate half of these substances using the recommended procedure for the isolation of basic drugs described earlier [25] without modification. For the remainder it was necessary to carry out some small but important changes to the method. In the case of pindolol, alprenolol and metoprolol it was necessary to transfer the supernatant directly onto the cartridge since the evaporation and reconstitution with 1 ml water caused a dramatic loss of drug. Since this at the same time represents a simplification of the method, it may be incorporated in the general procedure when possible.

For oxprenolol and mepindolol it was necessary to dilute the matrix (1 ml plasma) 5 times with water prior to the deproteinization step in order to improve the elution

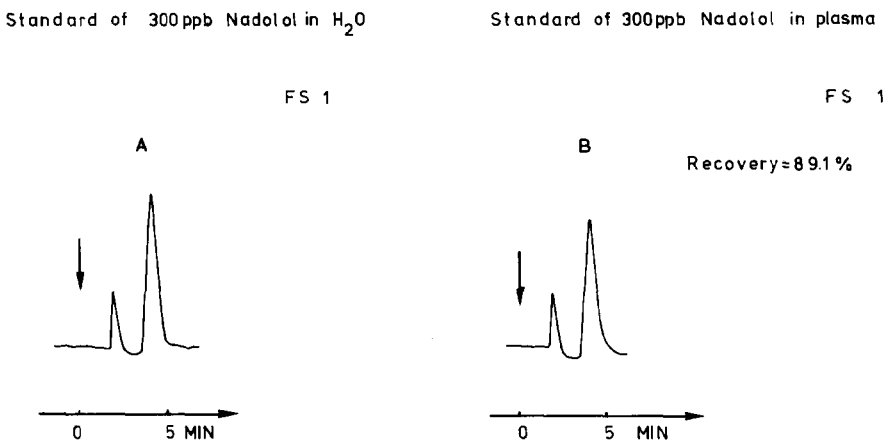

Figure 13
Chromatograms of nadolol: A, standard of 300 ng ml^{-1} nadolol in methanol–phosphate buffer; B, standard of 300 ppb nadolol in plasma. Fluorescence detection (230, 300) at a fixed scale of 1 was used.

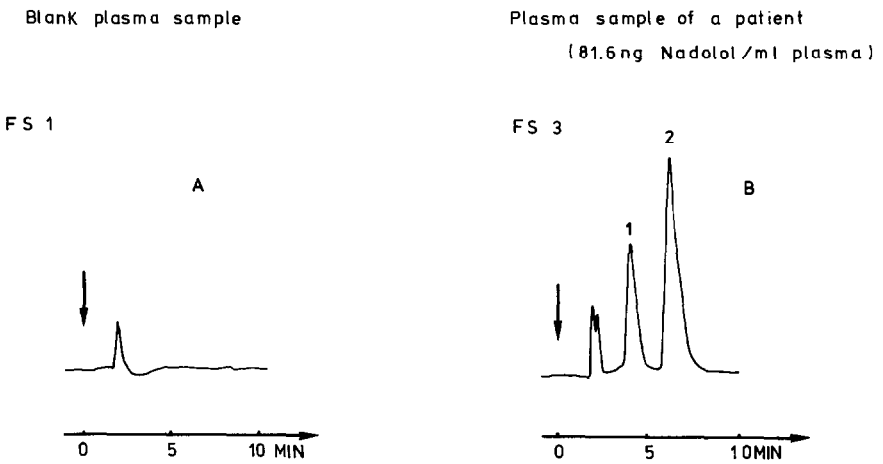

Figure 14
Assay of nadolol in plasma: A, blank plasma sample; B, plasma sample of a patient (81.6 ng nadolol/ml plasma (1) and 500 ng viloxazine/ml plasma (2) as internal standard. Fluorescence detection (230, 300) at a fixed scale of 3 was used.

recoveries. Finally, it was necessary to reduce the amount of plasma used to 200 μl in the case of sotalol. Another possibility is to use a larger amount of sorbent (3 or 6 ml capacity for instance), however, it is preferable if one type of cartridge is associated with the strategy, namely, CN 1 ml capacity.

For one compound of the drugs investigated, namely labetalol, the elution recovery given by the recommended procedure was not optimal. Perhaps a better recovery may be achieved by using a less apolar cartridge (C2) for instance.

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