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The determination of nadolol in biological samples using high-performance liquid chromatography

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Abstract: A method has been developed for the determination of nadolol in biological samples by reversed-phase high-performance liquid chromatography with fluorimetric detection. The method has been applied to plasma, serum and urine samples, which are prepared by extraction with diethyl ether–dichloromethane (5:2,v/v), evaporation of the organic solvent, and dissolution of the resultant residue in the chromatographic eluent. The sample is then subjected to chromatography on a C_{18}-silica column, with an eluent of water–acetonitrile–triethylamine (800:200:1,v/v) adjusted to pH 3.0 with orthophosphoric acid. A single point external standard is used for quantitation. The working ranges were 1–400 ng/ml for plasma/serum, and 0.1–40 μg/ml for urine, although a detection limit of 0.1 ng/ml appears to be readily attainable. The sample size was 0.5 ml, and for both types of sample the method showed good correlation with a previously published fluorimetric method (for plasma, $r = 0.9544$, $n = 70$; for urine, $r = 0.9919$, $n = 35$).

Keywords: *Nadolol; reversed-phase high-performance liquid chromatography; fluorimetric detection.*

Introduction

Nadolol is a long-acting beta-adrenoceptor antagonist [1]; in order to determine the drug in biological samples a reliable, sensitive and accurate assay is required. Nadolol has been determined in biological samples by a number of techniques. Ivashkiv [2] developed a fluorimetric method, which requires a comparatively large sample volume (for example, 2 ml of plasma or serum) and uses a time-consuming acid back-extraction and derivatization procedure, followed by fluorimetric quantitation. Although this method has been used satisfactorily in the author's laboratory on many hundreds of samples for over five years, a usually small but variable interference from endogenous components of the samples has been found in the assay, as observed by the original author [2]. Thus, the fluorimetric assay is adequate in many instances but is less than

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ideal for low sample volumes or low drug concentrations. Several other methods have been published subsequently, but these generally involve equipment or techniques which are not always readily available, for example the gas chromatography–mass spectrometric method of Funke [3], the HPLC method (with electrochemical detection) of Surmann [4], and the fluoridensitometric method of Schafer-Korting [5].

More recently, several workers have investigated the use of HPLC with either UV absorbance or fluorimetric detection; methods using both approaches are available for the determination of many other beta-adrenoceptor antagonists [6]. HPLC with UV detection has been used in the author's laboratory, but was found to be suitable only for samples such as urine or other fluids where comparatively large concentrations of nadolol could be anticipated. In this context, the approach has been applied to urine samples, serum samples where high nadolol concentrations were expected, and to buffer solutions from buccal absorption studies. Other workers have also followed this approach; Kinney [7] used it to determine the concentration of nadolol in plasma. The major problems were associated with the poor limit of quantitation. A realistic limit of detection is 10 ng nadolol/ml of sample, but to attain this limit the detector has to be set at a fairly high sensitivity; this leads to problems with baseline noise and drift, and the very real probability of interfering peaks from endogenous sample components. The problems with the baseline can be minimized by several techniques, including temperature control (or 'lagging') of the column, and re-circulating the eluent (as described by Kinney), but the author has been unable to remove the peaks from endogenous compounds satisfactorily.

The popular alternative HPLC approach is to use fluorimetric detection. A method has been described by Gupta and colleagues (personal communication, 1983), in which plasma samples are pretreated on a disposable BondElut® C_{18} column, and then subjected to chromatography on a resin-based HPLC column followed by detection and quantitation by fluorimetry. The method was used to determine concentrations down to 10 ng nadolol/ml plasma. Piotrovskii has also developed a procedure for nadolol using cation-exchange HPLC [8]. Although these methods would appear to be capable of modification to give the sensitivity and flexibility required in the present work, a more commonly available type of HPLC column was preferred, packed with a silica-based C_{18} phase. This is perhaps the single most widely used type of HPLC stationary phase, and as such is likely to be the most readily available.

This paper describes a reversed phase HPLC procedure with fluorimetric detection for the analysis of nadolol in biological samples. The method is simple, sensitive and accurate with good reproducibility and has the potential for at least partial automation.

Experimental

Reagents

Acetonitrile was HPLC grade (Rathburn Chemicals Ltd., Walkerburn, Peebleshire, UK). The water used was glass-distilled, and all other chemicals were obtained from BDH Chemicals Ltd (Poole, Dorset, UK) as follows: Decon 90 detergent (as supplied); triethylamine (reagent grade); sodium hydroxide (Aristar grade); orthophosphoric acid, potassium chloride, diethyl ether and dichloromethane (All AnalaR® grade).

All sample tubes were soaked overnight in a 5% aqueous solution of detergent (Decon 90); the tubes were then thoroughly rinsed with water, further rinsed with 0.3 M hydrochloric acid, finally rinsed with glass distilled water, and dried before use.

Chromatographic conditions

The HPLC system consisted of an Altex 110A pump, connected via a Wika pressure gauge to a Rheodyne 7125 injector, fitted with a 100- or 50-μl loop for plasma and urine assays, respectively. The analytical column was a 200 × 4.6 mm i.d. stainless steel column, slurry packed with 5-μm Hypersil ODS (Shandon Southern Products Ltd, Runcorn, Cheshire, UK). Other HPLC column packings were evaluated for use with this method. Apparently suitable alternatives were Zorbax ODS (DuPont) and Apex ODS (Jones Chromatography). The detector was a Perkin–Elmer model 204 spectrofluorimeter fitted with a flow cell (approximate volume 20 μl). The excitation wavelength was 265 nm, the emission being detected at 295 nm; the detector sensitivity was adjusted appropriately for the particular matrix being analysed (the detector 'sensitivity' was set at 9 and 4 for serum/plasma and urine respectively, and the 'selector' was set at ×10). The recorder was a Perkin–Elmer model 56, and was usually operated at 10 mV input and a speed of 0.5 cm/min. The mobile phase was prepared daily by mixing water and acetonitrile (4:1, v/v) then degassing under vacuum (700 mmHg) for 5 min. Triethylamine 1 ml/l was then added with mixing followed by adjustment of pH to 3.0 with orthophosphoric acid.

Preparation of standards

The nadolol stock solution contained 50 mg/100 ml in the mobile phase, and was kept for not more than 2 weeks at 4°C. A working standard was prepared daily by diluting an aliquot of the stock solution with an appropriate volume of mobile phase to a concentration of 200 ng/ml for use with serum/plasma samples, or 5 μg/ml for urine samples.

Preparation of sample

Samples that had been stored in the frozen state were thawed completely at ambient temperature and thoroughly mixed before analysis.

(a) *Plasma/serum samples*. The sample (0.5 ml) was transferred by pipette into a clean glass tube. Approximately 0.2 g of potassium chloride was added to the sample and dissolved by shaking. Diethyl ether:dichloromethane (5:2 v/v, 5 ml) was added followed by 0.5 ml of 5 M sodium hydroxide. The tube was stoppered and shaken gently by hand for 10 min. After the addition of sodium hydroxide the nadolol was extracted immediately (to minimize any degradation of the nadolol [2]); as soon as possible the tube was centrifuged at 3000 r.p.m. for 2 min. A 3-ml aliquot of the organic layer was transferred to a clean test tube and evaporated to dryness at 60°C with a rotary evaporator. The residue was dissolved in 300 μl of the mobile phase and 100 μl was injected on the column.

(b) *Urine samples*. The extraction procedure was identical to that described above, except that, in the final step, the residue was dissolved in 800 μl of the mobile phase and 50 μl was injected on the HPLC column.

Chromatography

Stable solvent flow and baseline conditions were obtained before injecting the appropriate standard solution; conditions were adjusted until reproducible chromatography was attained. The chromatography was checked each day by measuring the

retention and efficiency (number of theoretical plates) of the nadolol peak. Typical values were 5 min for the retention time, and an efficiency of 3000 theoretical plates. Figure 1 shows typical chromatograms.

Samples were quantitated by comparison of the peak heights of nadolol with those of the appropriate working standard.

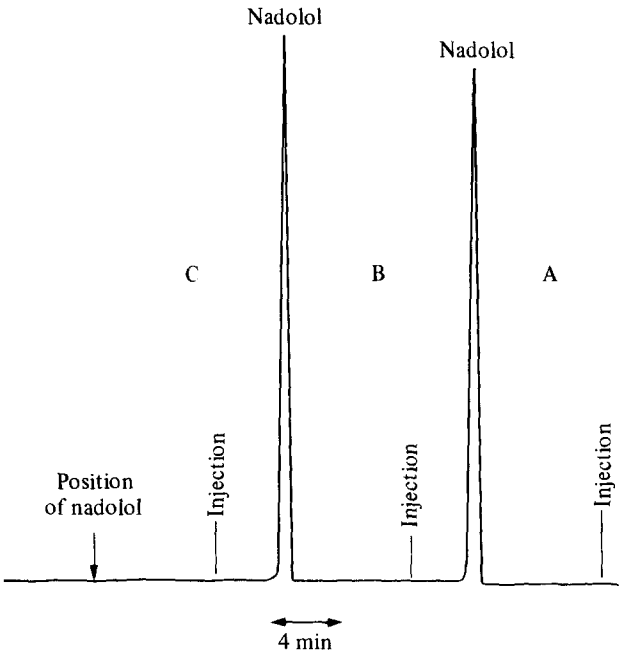

Figure 1
Chromatograms of nadolol in serum. (A) Standard nadolol solution in eluent (194 ng/ml). (B) Blank serum 'spiked' with nadolol (210 ng/ml). (C) Blank serum. Volume injected: 100 μl. For chromatographic conditions, see text.

Results and Discussion

Experiments were performed to determine the characteristics of the assay in terms of accuracy and precision.

Linearity of nadolol response

As a single standard is used for quantitation (instead of a calibration graph) it is essential that the relationship of the peak height of nadolol is linear with respect to the concentration of nadolol in the sample, over the concentration range used. The response (peak height in mm) of nadolol was determined over the ranges 40–300 ng/ml for plasma or serum (n = 6, 95% confidence limits of intercept -0.906 ± 1.305), and 0.5–10 μg/ml for urine (n = 5, 95% confidence limits of intercept -0.121 ± 0.629). Both responses were linear, as shown by the correlation coefficients (r) of 0.9999 in each instance.

The experiments were then repeated using various concentrations of nadolol in the appropriate matrix (serum and urine). Similar results were obtained, with linear correlation coefficients of 0.9998 in each instance, and intercepts of zero, within the 95% confidence limits (serum, n = 6, intercept -1.797 ± 1.879; urine, n = 5, intercept

-0.693 ± 0.893). Thus nadolol may be confidently determined in these matrices against a single standard.

Specificity

The procedure was applied to commercially obtained serum and to urine from a healthy subject to whom no drugs were being administered currently. No peak was observed at the retention time of nadolol in either instance.

Since the method may well be used to analyse samples from clinical studies where β-blockers other than nadolol may also be used, the possibility of interference in the assay by these compounds was investigated. Solutions of authentic materials were prepared in the mobile phase at approximately 200 ng/ml, and 50-μl aliquots were injected on the column.

The compounds, their elution times and their distribution coefficients [9] (determined in *n*-octanol/pH 7.0 phosphate buffer at 37°C) are given in Table 1.

Table 1
HPLC elution times and distribution coefficients (*n*-octanol/pH 7.0 phosphate buffer, 37°C) of various β-adrenoceptor blocking agents

Drug	Retention time (min)	Distribution coefficient
Atenolol	3.0	0.008
Sotalol	3.5	0.012
Nadolol	4.4	0.022
Pindolol	7.6	0.29
Acebutolol	n.o.*	0.35
Metoprolol	12.4	0.37
Timolol	n.o.*	0.51
Oxprenolol	n.o.*	1.01
Labetalol	n.o.*	8.3
Propranolol	n.o.*	8.6

*n.o., peak not observed.

The order of elution of the various compounds is clearly related to the distribution coefficients, and in general those compounds with larger values (acebutolol, timolol, oxprenolol, labetalol and propranolol) are not observed with this system. Thus no interference in the nadolol assay would be expected from the compounds examined. The possibility of using this procedure to determine the other compounds eluted (atenolol, sotalol, pindolol, metoprolol) was not investigated since HPLC methods appear to be readily available for these drugs [6]. The possibility exists also of using, for example, sotalol or pindolol as an internal standard, but again this was not investigated further.

Precision and accuracy

Nadolol was added to freeze-dried commercially obtained serum (James Turner (Liverpool) Ltd, Liverpool, UK) at three typical concentrations. These samples were then analysed in replicate ($n = 5$) by the method; the results are given in Table 2.

Nadolol was added to urine obtained from a healthy male subject (not fasted, not being administered nadolol, random time of sampling) at three typical concentrations.

Table 2
Absolute recovery of nadolol from serum at various concentrations

Nadolol concentration (ng/ml)	Absolute recovery		
	Mean (%)	RSD (%)	*n*
397	97	1.5	5
199	96	0.7	5
50	95	1.3	5

Table 3
Absolute recovery of nadolol from urine at various concentrations

Nadolol concentration (μg/ml)	Absolute recovery		
	Mean (%)	RSD (%)	*n*
30	98	0.7	5
15	96	2.4	5
7.5	96	1.9	5

These samples were then analysed by the method ($n = 5$); the results are given in Table 3.

Since the method uses no internal standard, it is essential that good recoveries and reproducibility are consistently obtained. The results confirm this with recoveries in the range 95–98% over the concentration range 0.05–30 μg/ml.

Detection limit

Under the conditions used, realistic limits of detection are 0.5 ng/ml for plasma/serum (50 pg injected on column), while the working range is 0.1–4.0 μg/ml for urine. However, the detection limit can readily be decreased to 0.1 ng/ml or lower: by increasing the sample size; by increasing the detector sensitivity; and by dissolving the dried sample residue in a smaller volume of mobile phase, and/or by increasing the injection volume.

Correlation of HPLC procedure with fluorimetric assay

In an attempt to gain additional information on the accuracy of the method, a number of plasma and urine samples from a clinical study were analysed by this method and by the original fluorimetric method. The correlation between the results obtained from the two methods is shown in Fig. 2 (plasma samples) and Fig. 3 (urine samples). The positive intercept (10 ng/ml) on the fluorimetric assay axis for the plasma samples is consistent with the previously mentioned interference from endogenous components in the sample. A similar situation is observed for the urine samples, although the positive intercept on the fluorimetric assay axis of 0.4 μg/ml is not statistically significant at the 95% level. Since the fluorimetric procedure has previously been correlated with a GC–MS method

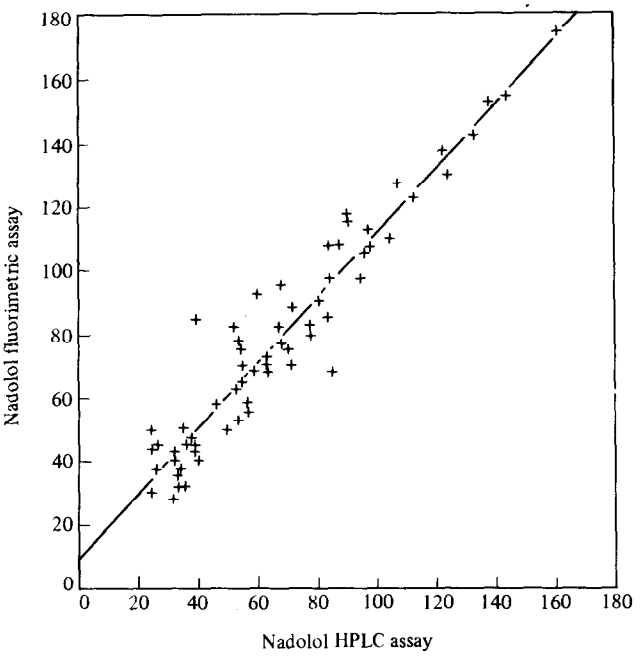

Figure 2
Correlation of HPLC method with fluorimetric assay for the determination of nadolol in human plasma. Gradient: 95% confidence limits = 1.0149 ± 0.0768. Intercept: 95% confidence limits = 9.5956 ± 5.5949. n= 70, r = 0.9544.

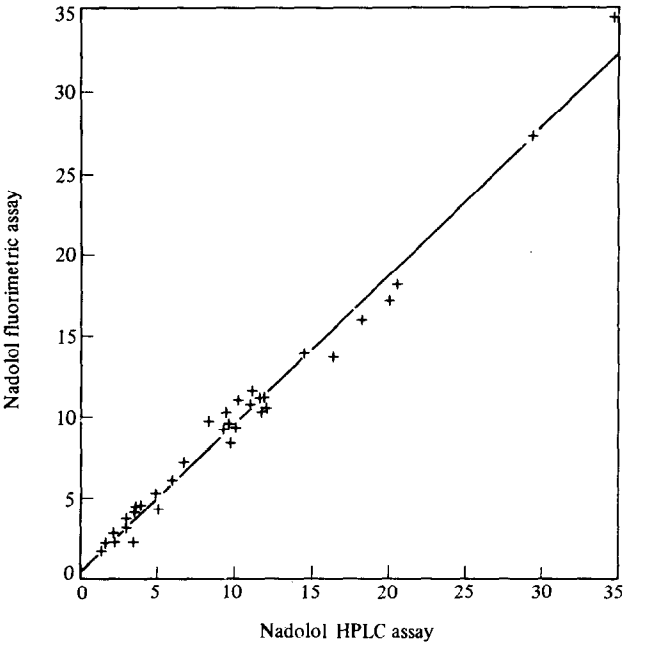

Figure 3
Correlation of HPLC method with fluorimetric assay for the determination of nadolol in human urine. Gradient: 95% confidence limits = 0.9155 ± 0.0413. Intercept: 95% confidence limits = 0.4692 ± 0.5060. n = 35, r = 0.9919.

[3], the HPLC procedure is shown to correlate well with both the fluorimetric method and (indirectly) with the GC–MS method.

Thus, this HPLC method offers a rapid, extremely sensitive and specific approach for the determination of nadolol in biological samples.

References

[1] J. Dreyfuss, D. L. Griffith, S. M. Singhvi, J. M. Shaw, J. J. Ross, R. A. Vukovich and D. A. Willard, *J. Clin. Pharmacol.* **19**, 712–720 (1979).
[2] E. Ivashkiv, *J. Pharm. Sci.* **66**, 1168–1172 (1977).
[3] P. T. Funke, M. F. Malley, E. Ivashkiv and A. I. Cohen, *J. Pharm. Sci.* **67**, 653–655 (1978).
[4] P. Surmann, *Arch. Pharm.* **313**, 1052–1054 (1980).
[5] M. Schafer-Korting and E. Mutschler, *J. Chromatogr.* **230**, 461–465 (1982).
[6] A. C. Mehta, *Pharm. J.* **230**, 191–194 (1983).
[7] C. D. Kinney, *J. Chromatogr.* **305**, 489–495 (1984).
[8] V. K. Piotrovskii, V. G. Belolipetskaya, A. R. El'mau and V. I. Metelitsa, *J. Chromatogr.* **278**, 460–474 (1983).
[9] P. B. Woods and M. L. Robinson, *J. Pharm. Pharmacol.* **33**, 172–173 (1981).

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