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Short Communication

Automated HPLC assay for sotalol in human plasma

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Introduction

Sotalol hydrochloride, N-[4-[1-hydroxy-2-[(1-methyl)amino]ethyl]phenyl]methan-sulphonamide hydrochloride (Fig. 1), is a hydrophilic non-selective beta-adrenoreceptor antagonist, which is used for the treatment of tachycardiac arrhythmia [1]. Several HPLC procedures have been used for the assay of sotalol hydrochloride in human plasma. Generally, C_{18} reversed-phase packings with mobile phases that contain methanol as modifier are used. Detection is achieved with UV-absorbance detectors [2, 3] or with fluorescence detection [4, 5]. Sample pre-treatment is done by fluid-extraction [3, 4], solid-phase extraction [2] or precipitation of plasma proteins [5]. The disadvantage of these methods is that they are time consuming and tedious. To manage the large number of analyses in pharmacokinetic studies, an automated HPLC assay based on a column-switching technique with on-line sample enrichment has been developed.

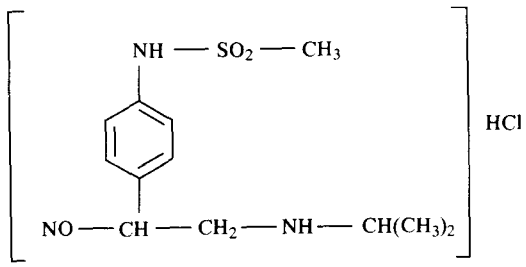

Figure 1
Structure of sotalol hydrochloride.

Experimental

Chemicals and reagents

The following reagents were used: acetonitrile LiChrosolv (Merck, Darmstadt, Germany); potassium dihydrogenphosphate p.a. (Merck, Darmstadt, Germany); dodecylsulphonic acid purum (Fluka, Neu-Ulm, Germany); 37–50-μm Bondapak Corasil C_{18} (Waters, Eschborn, Germany); 5-μm Shandon Hypersil, ODS (Grom, Ammerbuch, Germany); blank plasma (Blutzentrale, Ulm, Germany); HPLC-water prepared with MilliQ (Millipore/Waters, Eschborn, Germany); sotalol hydrochloride pharmaceutical quality (MIDAS Pharmachemie, Gau-Algesheim, Germany).

Instruments and conditions

Analytical separation. A pump with System Controller 600 E (Millipore/Waters) was used. The eluent was 0.01 M KH_2PO_4-buffer–acetonitrile (7:3, v/v) with 0.135% (w/v) of dodecylsulphonic acid and was adjusted to pH 6.0 with diluted H_3PO_4 or KOH and degassed. The flow-rate was 1.0 ml min^{-1}. The HPLC column (Hyperchrome 125 × 4.6 mm i.d.) was filled with 5-μm Shandon ODS (home made). The column temperature was 35°C. The tunable absorbance detector 486 (Millipore/Waters) was set to 227 nm. For data handling and integration, a Millennium Chromatography Manager 2010 (Millipore/Waters) was used with an external standard method. The total analysis time was 13 min.

Sample enrichment. A Kontron LC pump 414 T (Kontron, Eching, Germany) was used.

The eluent was HPLC water and the washing period was 10 min. The flow-rate was 1.0 ml min^{-1}. Wisp 712 (Millipore/Waters) sampler was used with a sample volume of 500 µl. The precolumn was Hyperchrome (40 × 4.6 mm i.d.) and was dry-filled with 37–50-µm Bondapak Corasil C_{18}. The switching valve was a Valco 10-port-multi-functional valve with an air actuator (Chrompack, Frankfurt/M, Germany) and was timed via time events from the System Controller 600 E.

Calibration and control samples

Calibration samples of sotalol hydrochloride were prepared with water by dilution to yield solutions of 10, 60, 120, 240 and 600 ng ml^{-1}, respectively. Calibration was achieved using the linear regression least-squares method. In the assay development a concentration of 1200 ng ml^{-1} was also included. During the determination of plasma samples in a bioequivalence study, however, this concentration proved to be too high so it was omitted for routine work. Control samples were prepared from an aqueous stock solution with blank plasma in the last two dilution steps to yield 60, 240 and 600 ng ml^{-1} sotalol hydrochloride. The control samples were frozen, stored and handled like the other samples of a series.

Sample preparation

The frozen plasma or control samples were thawed at 37°C and centrifuged at 5000 rpm for 5 min. One millilitre of the clear supernatant fluid was diluted with 1.0 ml of water in the sampler vials by shaking vigorously. Each sample was analysed in duplicate.

Chromatographic procedure

The HPLC apparatus is illustrated in Fig. 2. The sample was injected into the solvent flow from pump B using the autosampler for concentration on the first conditioned precolumn, with water as the solvent. After 10 min the valve was switched to the alternate position and the absorbed material was flushed back to the analytical column with the stronger eluent from pump A. During the separation time the next precolumn was conditioned with water and the next sample was loaded for preconcentration. After the washing time this sample was introduced into the flow of pump A for HPLC separation.

Results and Discussion

Assay development

The first attempts were made using separation systems found in published work (see

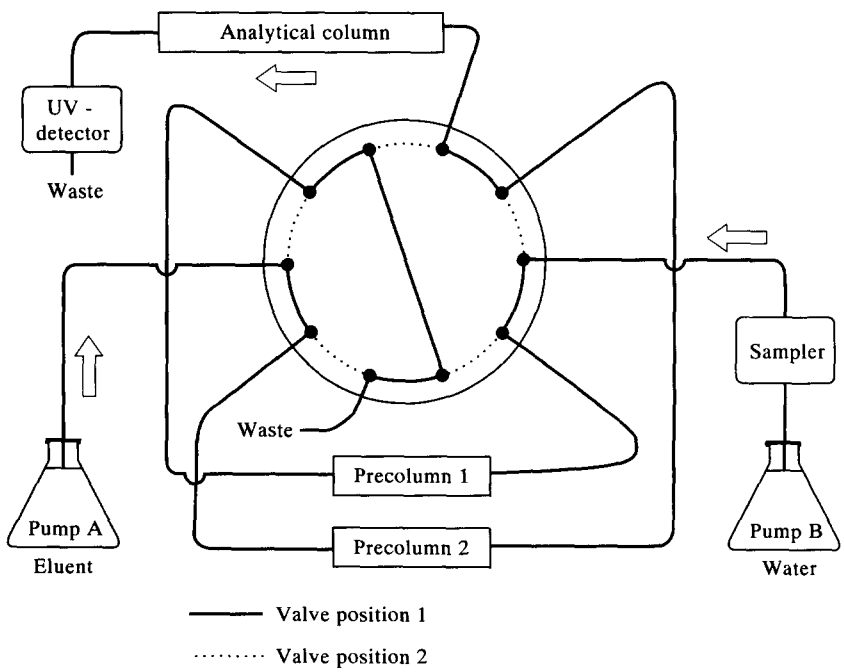

Figure 2
Diagram of the HPLC apparatus for direct sample enrichment.

references). Owing to the polar nature of the analyte, however, rather weak eluents are commonly used. This resulted in poor separation of endogenous substances and accumulation of these substancess on the enrichment columns. To overcome these problems, dodecylsulphonic acid was added to the analytical eluent as an ion-pairing reagent with a rather long alkylchain to retain the analyte for a longer period. Thus it was possible to use a stronger eluent as basis for the separation, which resulted in trouble-free work. Over continuing series of plasma samples, no additional cleaning or reconditioning of the columns was necessary.

Method validation

Linear range. After the oral administration of 80 mg of sotalol hydrochloride maximal concentrations up to 600 ng ml^{-1} in plasma were expected. For higher peaks the assay development was extended to the range 10 ng ml^{-1}–1.200 ng ml^{-1} (Table 1).

Limit of quantitation. The limit of quantitation was calculated from the confidence interval of the inverse of the linear calibration function. It is the lowest mass to be differentiated from zero mass with a present probability of 5% error [6]. The basic model was linear regression without weighting factors. The limit of quantitation was determined to be 10.0 ng ml^{-1}.

Recovery. The recovery for the assay was determined with spiked plasma samples. It was calculated from 10 replicate measurements over the whole linear concentration range (Table 2).

Table 1
Linear range of calibration graph for sotalol hydrochloride

Range (ng ml^{-1})	10–1.200
Slope (ng ml^{-1} $Area^{-1}$)	553.1
Intercept (Area)	−2270
Correlation r^2	0.9987
Number of measurements	64

Precision. The precision of the method was determined as repeatability in one series over the whole concentration range that was covered by the linear calibration function (Table 2). The reproducibility from day to day was calculated from the measurement of control samples in the relevant concentration range in a study lasting 4 weeks (Table 3).

Selectivity. The selectivity of the analysis with respect to endogenous substances is shown by the comparison of a pre-dose sample and a sample 4 h after the administration of 80 mg sotalol hydrochloride to a volunteer (Fig. 3). There was no deterioration of the sotalol peak by other compounds. This was also true for samples from a bioavailability study with 16 volunteers.

Stability of samples. Plasma samples with known concentrations of sotalol hydrochloride were frozen and stored over a period of 8 weeks. No changes in concentration were observed.

Pharmacokinetic study. In a bioavailability study [7] with 16 healthy volunteers more than 600 plasma samples were measured. Together with calibration and control samples this amounted to about 800 injections. The analyses were performed without any problems, such as clogging of columns or influence of endogenous substances on the chromatographic separation. As a precaution, precolumns were changed every 120 injections. An example of a time vs concentration profile following the administration of 80 mg sotalol hydrochloride as a tablet is given in Fig. 4.

Conclusions

A rapid, sensitive and selective HPLC method has been developed for the assay of sotalol hydrochloride in human plasma. The analysis is automated by column switching and on-line enrichment on two precolumns to such an extent that dilution is the only manual step of pre-treatment on the centrifuged sample.

Table 2
Recovery and repeatability in a series of determinations of sotalol hydrochloride

Concentration (ng ml^{-1})	1200	600	240	60	10
Recovery (%)	102.4	101.3	98.5	94.5	96.4
RSD (%)	1.17	1.16	0.78	1.53	13.61
Number of measurements	10	10	10	10	10

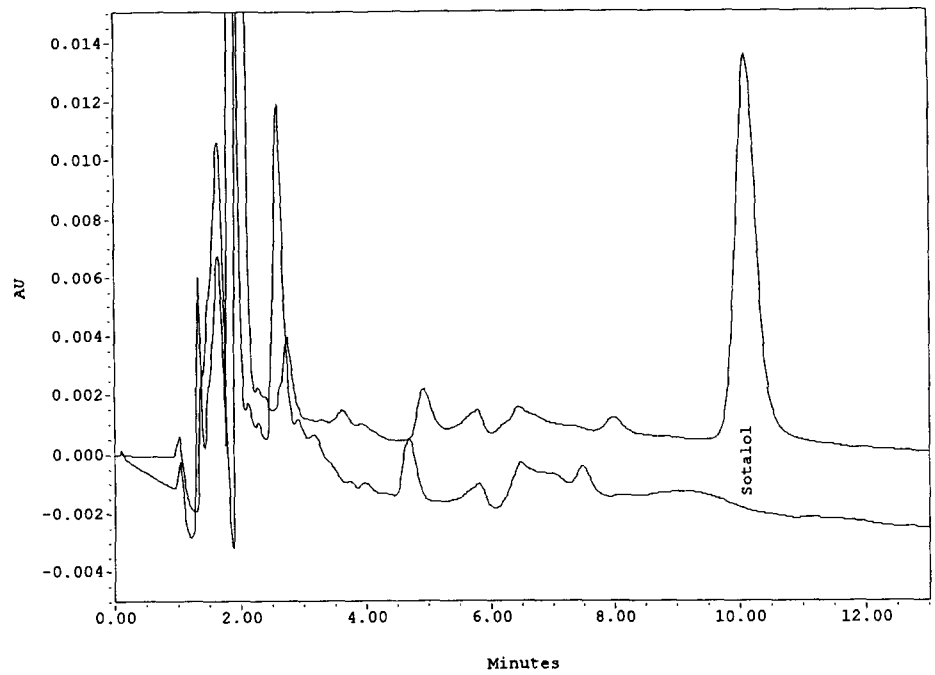

Figure 3
Chromatograms of plasma samples: lower trace-blank plasma; upper trace-plasma containing about 600 ng ml^{-1} of sotalol hydrochloride.

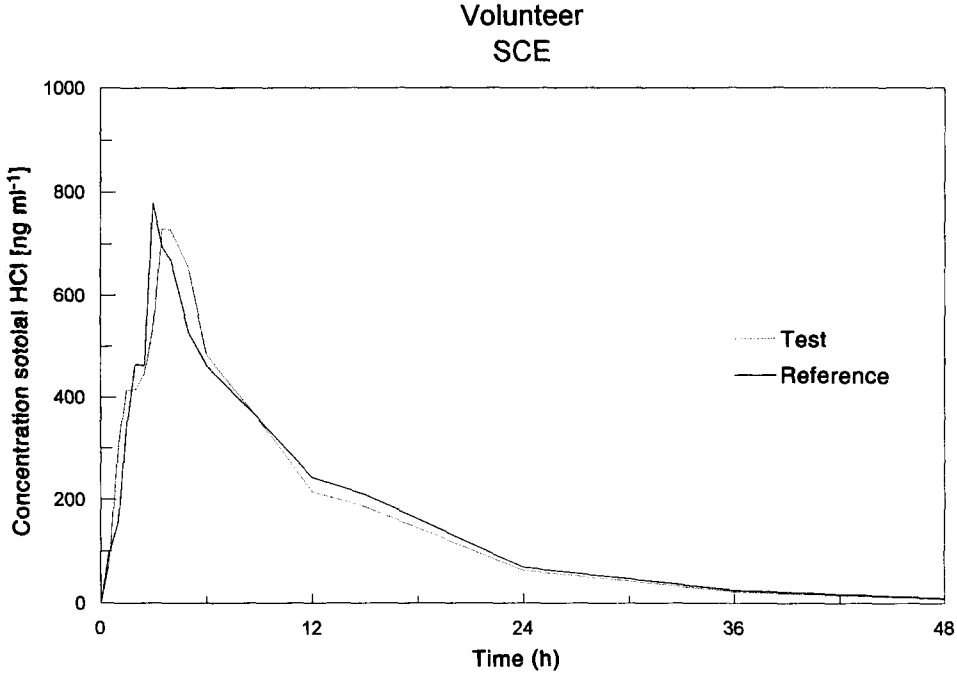

Figure 4
Sotalol concentration versus time graph after administration of 80 mg of sotalol hydrochloride as tablets in a test and reference preparation, respectively.

Table 3
Recovery and reproducibility from day to day of control samples of sotalol hydrochloride

Concentration (ng ml^{-1})	600	240	60
Recovery (%)	101.1	100.3	92.8
RSD (%)	5.24	10.9	13.0
Number of measurements	29	30	27

The method shows nearly complete recovery of the analyte and good precision in the linear range 10–1200 ng ml^{-1}. The method was used for a pharmacokinetic study and proved very robust over a series of more than 800 chromatograms.

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References

[1] A. Fitton and E.M. Sorkin, *Drugs* **46**, 678–719 (1993).
[2] G.L. Hoyer, *J. Chromatogr.* **427**, 181–187 (1988).
[3] R. Urech, L. Chan and P. Duffy, *J. Chromatogr.* **534**, 271–278 (1990).
[4] M.J. Bartek, M. Vekshteyn, M.P. Boarman and D.G. Gallo, *J. Chromatogr.* **421**, 309–318 (1987).
[5] J. Boutagy and G.M. Shenfield, *J. Chromatogr.* **565**, 523–528 (1991).
[6] S. Ebel, H. Kühnert and W. Mück, *Chromatographia* **23**, 934–938 (1987).
[7] R. Herrmann and C.H. Kleinbloesem, *Arzneim.-Forsch.* **44**, 589–592 (1994).

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