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Automatic solid-phase extraction and high-performance liquid chromatographic determination of quinidine in plasma

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Abstract

High-performance liquid chromatography (HPLC) was used for the therapeutic drug monitoring of quinidine in clinical samples. Solid-phase extraction (SPE) was studied in both off-line and on-line modes. SPE was performed in an automatic on-line mode using a fully automated Prospekt system. Extraction recoveries were in the range 97.1–99.4% for 1–2 μg/ml quinidine concentrations. For HPLC separation an Ultrasep RP-8 reversed-phase column was applied with acetonitrile–water (9:1) containing 0.3% triethylamine (pH 2.5) as the mobile phase. The Prospekt system is recommended for the routine monitoring of quinidine in plasma samples. Concentrations were in therapeutic range (1.2–3.6 μg/ml).

1. Introduction

Quinidine is widely used in the therapy of atrial fibrillation and certain other cardiac arrhythmias. It is generally regarded as a myocardial depressant drug as it lowers excitability, conduction velocity and contractibility. The quinidine preparations given to patients contain small amounts of dihydroquinidine, which has cardiac effects similar to those of quinidine [1].

Several different assay methods for quinidine have been described. Such as fluorescence measurements, which are non-specific. Certain high-performance liquid chromatographic (HPLC) procedures have been published for quinidine determination in therapeutic concentrations in plasma (2–5 μg/ml).

Liquid extraction with benzene has been applied for the simultaneous determination of quinidine and caffeine with theobromine as an internal standard. The detection limit was 0.5 μg/ml with UV detection (254 nm) [2]. A cyano-bonded column has been tested for the HPLC determination of quinidine in serum and urine [3]. Both UV and fluorescence detection were compared.

Quinidine and quinine have been determined in sheep plasma samples following extraction with hexane–ethyl acetate (9:1). The main pharmacokinetic parameters were calculated [4]. Standard extraction procedures have been published for a general method and the analysis of pharmaceutical dosage forms by HPLC using a μBondapak C_{18} column with binary solvent system has been reported [5].

An HPLC method for some indole and quinoline alkaloids including quinidine and quinine used a LiChrosorb RP-8 Select B column and UV detection (275 nm) [6]. HPLC with fluorescence detection has been recommended

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for the determination of quinidine and quinine in plasma and blood following simple liquid–liquid extraction; cinchonine was applied as an internal standard. The extraction recoveries were 79.3% for quinidine and 82.9% for quinine [7].

Extraction with methanol–dichlormethane (15:95) has been applied to the HPLC separation and determination of quinidine in serum with UV detection at 210 nm [8]. The calibration graph was linear in the range 1–5 μg/ml.

The aim of this work was to develop a rapid and simple method for the automated on-line SPE–HPLC determination of quinidine in plasma samples for the therapeutic concentration monitoring of patients treated with quinidine as an active antiarrhythmics. This method could permit analysis without a separate plasma preseparation and direct injection of clinical samples is possible.

2. Experimental

2.1. Apparatus

HPLC experiments were carried out on an LKB (Bromma, Sweden) modular HPLC system and a Knauer (Bad Homburg, Germany) isocratic HPLC system. For automatic sample preparation, the Prospekt SPE system (SunChrom Friedrichsdorf, Germany) combined with a solvent delivery unit (SDU) and a Marathon autosampler was tested. SPE Bakerbond cartridges (C_{18}, 10 × 3 mm I.D.) (J.T. Baker, Gross-Gerau, Germany) were used for on-line SPE of clinical samples. The mobile phase was acetonitrile–water (15:85) containing 0.3% of triethylamine (pH 2.5) at a flow-rate of 0.6 ml/min. Phosphoric acid was used to adjust the pH to 2.5. Separations were carried out with an Ultrasep C_8 column (Bischoff, Leonberg, Germany).

2.2. Chemicals

Standards of quinidine and quinine were obtained from Slovakofarma (Hlohovec, Slovak Republic) and methanol, acetonitrile, triethylamine and phosphoric acid were supplied by Lachema (Brno, Czech Republic).

2.3. Procedure

In the on-line SPE mode, the cartridge was washed with methanol and with water, each for 2 min (2 ml/min). After loading a clinical sample (100 μl), the proteins were removed by washing with water for 2 min (1 ml/min) and the drugs were eluted into the chromatographic column with the mobile phase for 1 min (0.6 ml/min).

3. Results and discussion

According to the literature, the recommended content of acetonitrile in the mobile phase is 4–20% (v/v). Triethylamine is an important component of the mobile phase as it improves the peak shape, resolution and selectivity. It was found that its concentration did not change the retention time significantly. 0.3% of triethylamine was sufficient and with 15% acetonitrile yielded a resolution $R_{ij} = 1.4$ and a selectivity coefficient of 1.25.

Automatic on-line SPE is a very effective preseparation technique and coupling the preseparation cartridge with the analytical column gives the possibility of direct injection of clinical samples. This procedure was realized using the Prospekt system, which is a fully automated on-line sample clean-up and injection system that combines the constant quality assurance advantages of a disposable cartridge system with the automated aspects of precolumn technology [9]. Samples are automatically loaded on disposable cartridges, purged with one or more liquids for sample clean-up and directly eluted to the HPLC column (Fig. 1). Within the Prospekt system all samples undergo exactly the same procedure including the time span between extraction and chromatographic separation. The whole system is controlled by the Prospekt unit to change solvents, flow-rates and number of steps. 99 programmes give the possibility of changing the parameters for different types of analyses. A

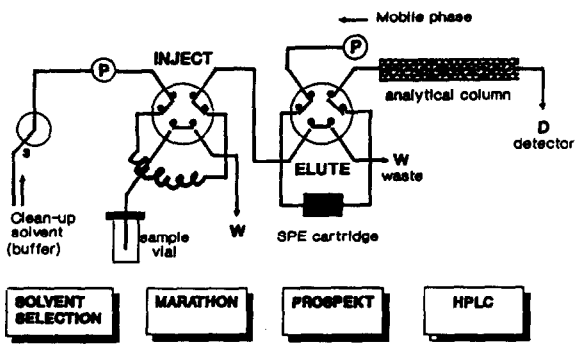

Fig. 1. Switching diagram for elution and analysis.

schematic diagram of the SPE–HPLC system is shown in Fig. 2.

A chromatogram obtained by automatic SPE–HPLC analysis of a serum sample (after treatment with quinidine) with the quinine as an internal standard, using the Bischoff C_8 column and the Prospekt system with Bakerbond cartridges, is illustrated in Fig. 3. Both compounds and dihydroquinidine were well separated. No interferences from serum sample are revealed in chromatogram.

The detection limit for quinidine was 25 ng/ml and the extraction recoveries were 97.1–99.4% for 1–2 μg/ml quinidine concentrations.

The method has been applied in hospital laboratories for routine monitoring. The overall procedure is efficient as the system begins the analysis of a new sample before that of the previous sample is completed. The results of some practical clinical analyses are given in

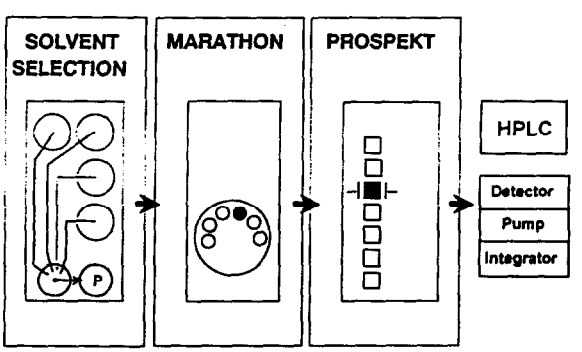

Fig. 2. Fully automated sample preparation system.

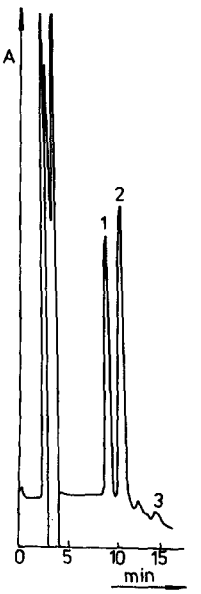

Fig. 3. SPE–HPLC of a clinical sample. Column, Bischoff Ultrasep C_8; Mobile phase, acetonitrile–water (15:85) containing 0.3% triethylamine (pH 2.5); flow-rate, 0.6 ml/min; detection, UV at 250 nm. Peaks: 1 = quinidine; 2 = quinine; 3 = dihydroquinidine.

Table 1. All the quinidine concentrations were within the therapeutic range.

4. Acknowledgements

We thank SunChrom (Spark Deutschland), (Friedrichsdorf, Germany) for providing the Prospekt system and Donau Trading (Zürich, Switzerland and Bratislava, Slovakia) for the Bischoff Ultrasep C_8 column. Bakerbond car-

Table I
Quinidine concentrations in clinical samples

Patient No.	Quinidine (μg/ml)	Within-batch R.S.D. (%) ($n = 5$)
1	3.4	1.4
2	3.3	1.8
3	3.6	2.1
4	1.2	1.2
5	2.3	1.9

tridges were supplied by J.T. Baker (Gross-Gerau, Germany).

5. References

[1] *Drug Monitoring Data*, American Association for Clinical Chemistry, Washington, DC, 1980.
[2] L.N. Ace and B. Chandhuri, *Anal. Lett.*, 20 (1987) 1483.
[3] J.J. McKichan and B. Shields, *Ther. Drug Monit.*, 9 (1987) 104.
[4] G.W. Mikaly, K.M. Hyman and R.A. Smallwood, *J. Chromatogr.*, 415 (1987) 177.
[5] A.S. Sidhu, J.M. Kennedy and S. Deeble, *J. Chromatogr.*, 391 (1987) 233.
[6] A.L. Hermans, T. Van der Leer and R. Verpoorte, *J. Chromatogr.*, 479 (1989) 39.
[7] M.D. Edstein, E. Prasitthipayoung and A. Sabchareon, *Ther. Drug Monit.*, 12 (1990) 493.
[8] G.L. Hoger, D.C. Clawson and A. Brookshier, *J. Chromatogr.*, 572 (1991) 159.
[9] *Prospekt, Technical Specifications*, Spark Holland, Emmen.