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On-line solid-phase extraction of piroxicam prior to its determination by high-performance liquid chromatography

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ABSTRACT

A direct method for the determination of piroxicam in plasma is described. Plasma is directly injected onto the extraction column (10 mm × 2 mm I.D., packed with 40-μm Bond Elut C_2) where piroxicam is separated from the plasma concomitants using a solid-phase extraction procedure. Using a laboratory-made on-line column-switching system, the drug is quantitatively transferred and separated on the analytical column (15 cm × 4.6 mm I.D., Supelcosil LC18 DB, 5 μm) followed by determination using ultraviolet absorption at 331 nm. Validation of the method demonstrated a good recovery (100%), sensitivity (limit of determination 0.2 μg/ml, based on a 20-μl sample volume), accuracy and precision (better than 5%). The developed method has been adopted for studying the steady-state pharmacokinetics of the drug.

INTRODUCTION

The therapeutic application of non-steroidal anti-inflammatory drugs (NSAIDs) is widespread in relieving arthritis. The therapeutic benefits of piroxicam in rheumatoid and osteo-arthritis are well documented [1,2].

During phase 1 clinical trials and bio-equivalence studies, there is a need for analytical methods for the accurate determination of the concentration of drugs in plasma. High-performance liquid chromatography (HPLC) is one of the most extensively and frequently used analytical techniques for determinining the concentration of piroxicam in plasma [3–7]. The method usually consists in the isolation of the drug from interfering plasma concomitants using liquid–liquid extraction prior to HPLC determination. The liquid–liquid extraction procedure concentrates the drug and therefore improves the detection limit. The extraction procedure is prone to complications because it involves several separate steps, which not only make the method tedious and time-consuming but also increase the potential of introducing a bias in the results. Therefore, extraction procedures which eliminate manual sample manipulation must be developed.

The on-line solid-phase extraction, separation and quantitation of drugs in

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complex sample matrices such as plasma is gaining widespread acceptance and application [8,9]. Using this approach, a rapid, direct, accurate and precise method has been developed for the determination of piroxicam in plasma. No sample pretreatment is necessary. The proposed method has demonstrated a 100% recovery at three different concentrations, and repeatability and reproducibility in terms of the coefficient of variation are better than 5%. Using a 20-μl sample volume, the limits of determination and detection are 0.2 and 0.1 μg/ml, respectively. The method is suitable for drug level monitoring and pharmacokinetic studies.

EXPERIMENTAL

Chemicals and reagents

USP piroxicam reference standard was purchased from USPC (Rockville, MD, U.S.A.). Acetonitrile (HPLC grade) was delivered by Rathburn (Walkerburn, U.K.). Dihydrogen sodiumphosphate, disodium hydrogenphosphate, sodium hydroxide and triethylamine were supplied by E. Merck (Darmstadt, Germany).

Preparation of standards

A stock standard solution of piroxicam (2.5 mg/ml) was prepared in 0.1 *M* sodium hydroxide solution. This solution was further diluted 1:1 with distilled water and was then used for the preparation of working standards containing 50, 250, 500 and 750 μg piroxicam per ml. Spiked plasma standards for the calibration graph ranging from 1.0 to 25 μg/ml and three quality control standards of 1, 5 and 15 μg/ml piroxicam were prepared from the working standards. Some spiked plasma samples were also prepared containing less than 1.0 μg/ml piroxicam for studies of the limits of determination and detection. The stock standard solution was stored at 4°C; the drug-free plasma and spiked plasma standards were kept at −20°C until analysis.

On-line solid-phase extraction

The HPLC system consisted of a Spectra Physics (San Jose, CA, U.S.A.) Model SP-4290 integrator equipped with a Model SP-8800 programmable solvent-delivery system, a Model SP-8780 sample processor for automated injection and a Model SP-200 programmable ultraviolet detector. A Spectra Physics Model 3500 pump was used as an auxiliary pump. Both pumps were operated at a flow-rate of 1.0 ml/min. On-line switching between the extraction and the analytical column was achieved with a laboratory-made, fully automated column-switching station (FACS). The built-in electronic components of the FACS allowed communication with an IBM PS/2 to automate the entire analysis procedure. A four-step diagram for the determination of piroxicam in plasma involving extraction, retention, elution and preparation using FACS is shown in Fig. 1. The effluent from the analytical column was monitored at 331 nm.

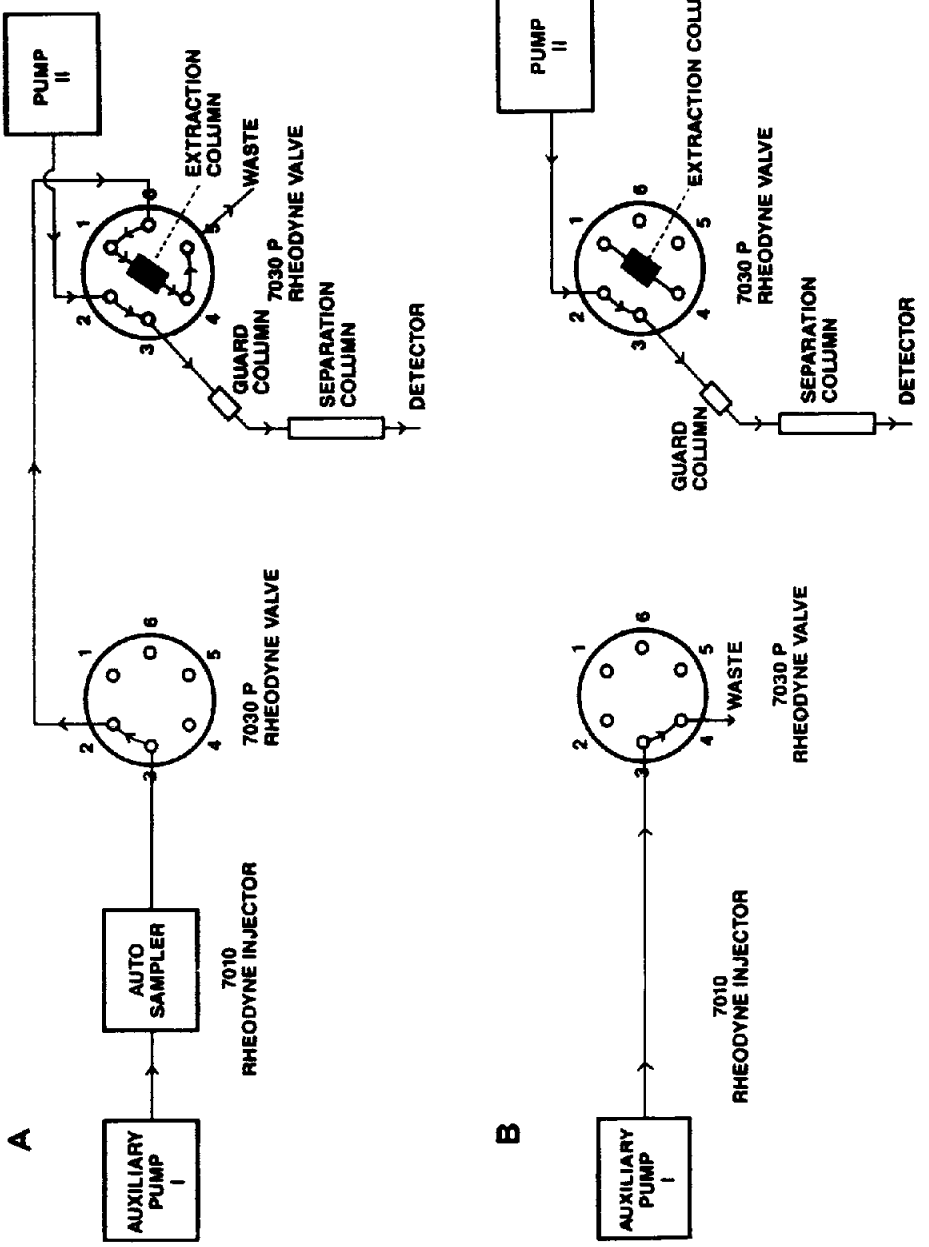

Fig. 1.

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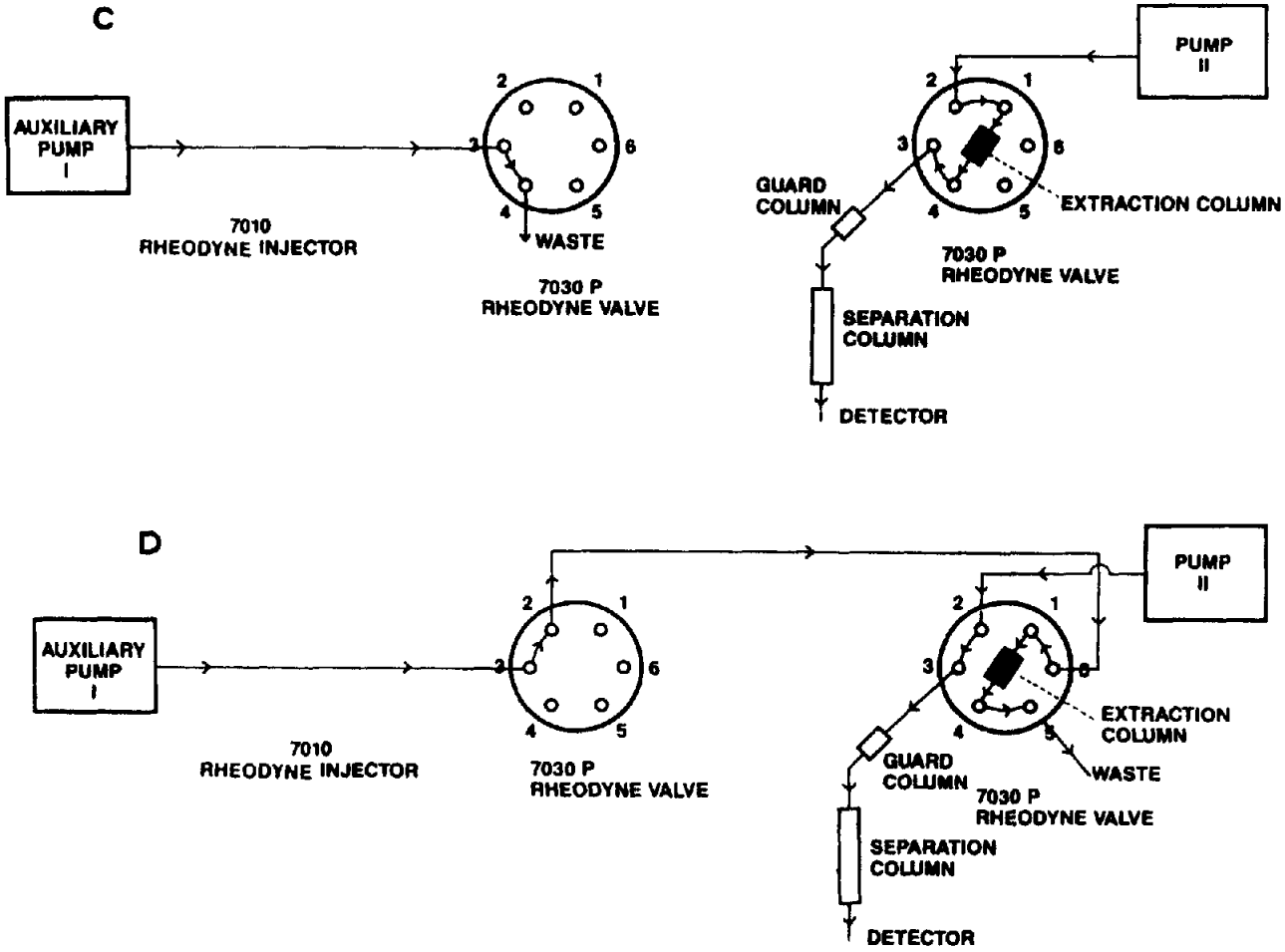

Fig. 1. On-line column switching using laboratory-made FACS system. (A) Injection and extraction of plasma sample: (B) residence of drug on the extraction column; (C); separation of the drug; (D) conditioning of extraction column.

Spectra Physics Labnet/Chromnet HPLC software (Winner 386), installed on an IBM PS/2, fully programmed the FACS and HPLC systems, and carried out the storage and retrieval of all chromatographic data.

The extraction column consisted of a Chrompack cartridge (Middelburg, The Netherlands) equipped with a cartridge column (10 mm × 2 mm I.D.). The column was packed manually with 40 μm particle size Bond Elut C_2 (functional group: ethyl, two-carbon straight-chain hydrocarbon) manufactured by Analytichem International (Harbor City, CA, U.S.A.). Each extraction column was conditioned prior to use in the following order: acetonitrile 100%, water and 0.15 *M* sodium dihydrogenphosphate, pH 3.5, adjusted with orthophosphoric acid (mobile phase 1).

The analytical column consisted of a Supelcosil LC18 DB column, (5 μm, 15 cm × 4.6 mm I.D.) interfaced with a Supelcosil LC18 DB (2 cm × 4.6 mm I.D.) guard column (Supelco, Bellefonte, PA, U.S.A.).

Extraction and separation of piroxicam using the fully automated column-switching station

Aliquots of plasma sample (20 μl) were transported to the extraction column using an auxiliary pump. Mobile phase 1, which passed through the column and was directed to waste, removed the bulk of the plasma matrix while quantitatively retaining piroxicam on the stationary phase (Fig. 1A). After 1 min, the FACS switched mobile phase 1 to drain. At this stage, the residual contents of the plasma were left intact on the extraction column for 1 min without any solvent flow (Fig. 1B). Until this stage, mobile phase 2 [0.02 *M* disodium hydrogenphosphate containing 5.10^{-4} *M* triethylamine pH adjusted to 3.1 with orthophosphoric acid–acetonitrile (40:60, v/v)] passed straight through the analytical column. After 2 min the FACS switched the system to straight-flush the adsorbed contents of the extraction column into the analytical column with mobile phase 2 (Fig. 1C). After 3 min mobile phase 1 was switched back to pass through the extraction column to prepare it for next sample, while pump II maintained the flow of mobile phase 2 through the analytical column (Fig. 1D). A new sample was injected after 8 min. Both the extraction and the analytical columns were maintained at ambient temperature.

RESULTS AND DISCUSSION

Optimisation

During the extraction process when the bulk of the plasma matrix is removed, it was suggested that the drug should be left on the sorbent without any solvent flow to improve the injection characteristic on the analytical column. In a series of experiments it was demonstrated that a residence time of 1 min not only increased the sampling efficiency but also enhanced the chromatographic profile, resulting in improved symmetry and low tailing. Packing material of C_2 was preferred to C_8 as it gave good and improved piroxicam chromatograms.

As a result of its strong protein-binding and pK_a value, piroxicam requires an acidic mobile phase for extraction. Several phosphate buffers of various concentrations in pH range 3–4 were investigated with and without organic modifiers (acetonitrile 5–10%, v/v). Phosphate buffer (0.15 *M*) at pH 3.5 without any organic modifier gave the optimum extraction. In mobile phase 2, however, the concentration of acetonitrile had to be raised to 60% (v/v) to achieve the quantitative transport of piroxicam from the extraction to the analytical column.

Fig. 2. shows chromatograms of blank plasma, plasma spiked with piroxicam and a plasma sample from a healthy volunteer who participated in the steady-state study of piroxicam. No endogenous interfering peaks were visible in the blank plasma. There is, however, a peak with a retention time of 4.80 min in Fig. 2c (authentic sample) which is not present in the spiked plasma (Fig. 2a). A

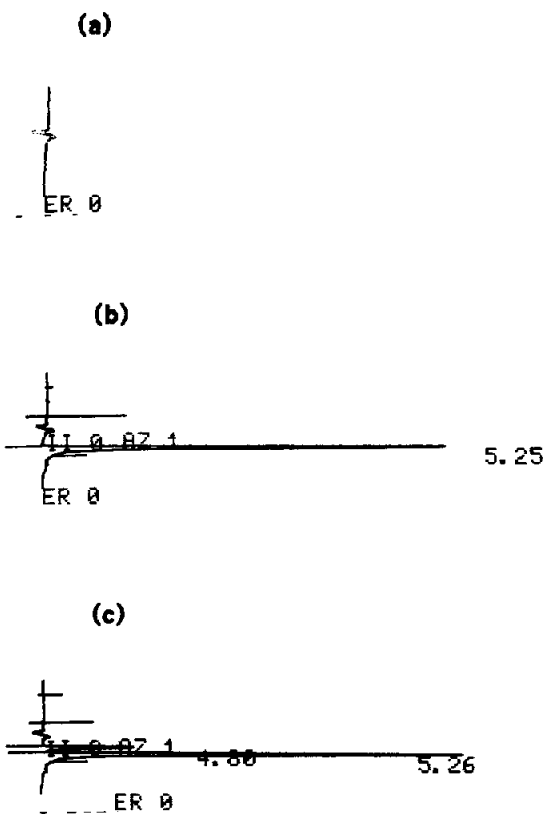

Fig. 2. Chromatograms of (a) drug-free plasma, (b) drug-free plasma spiked with 10 μg/ml piroxicam standard and (c) plasma sample from a patient containing 10.1 μg/ml piroxicam after injection of 20 μl of plasma and column switching. Extraction column, Bond Elut C_2; analytical column, Supelcosil LC18; mobile phase for extraction, 0.15 *M* NaH_2PO_4, pH 3.5; mobile phase for separation, 0.02 *M* Na_2HPO_4 containing 5.10^{-4} *M* triethylamine, pH 3.1–acetonitrile (40:60, v/v); detector, ultraviolet, 331 nm, attenuation, 16.

comparison of the ultraviolet spectrum obtained with a diode array detector of this peak and the piroxicam peak revealed a close resemblance in molecular absorption characteristics. The peak could be 5'-hydroxypiroxicam, the major metabolite of piroxicam [5,7]. No further experiments were carried out during these studies to identify this peak.

Validation

Linearity. Blank plasma spiked with 0, 1, 5, 10, 15 and 25 μg of piroxicam per ml were analysed on several days using the proposed method. The calibration graphs constructed by plotting the peak area *versus* concentration demonstrated an excellent linearity ($r^2 = 0.9995$) over the entire range tested. On average, the slope of the calibration graph obtained on six different days did not vary by more than 2%, demonstrating the good stability of the measuring system. This may indicate that it is not necessary to analyse all of the standards every time; however, it may be advisable to run one or two standards to check the response of the measuring system.

Recovery. The percentage recovery of piroxicam using the proposed procedure was studied by comparing the results obtained after the injection of plasma spiked with known concentrations with that produced by the same concentration of drug dissolved in aqueous medium. According to results obtained, the recovery is 95% at low (1 μg/ml) and 97.3% at high (25 μg/ml) concentrations.

Accuracy. Once the quantative recovery of piroxicam in plasma was established, the accuracy of the method was assessed using blank plasma spiked with three different concentrations of drug. The samples were pooled and stored at −20°C in 3–4 ml of polyethylene. These samples were always analysed together with normal plasma samples. A calibration graph was plotted based on plasma standards and the concentrations of three spiked samples were calculated using this graph. The results given in Table I show that on three different occasions the

TABLE I

ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF PIROXICAM USING THE PROPOSED METHOD

Piroxicam added (μg/ml)	Piroxicam found (n = 6)					
	Run 1		Run 2		Run 3	
	Mean (μg/ml)	R.S.D. (%)	Mean (μg/ml)	R.S.D. (%)	Mean (μg/ml)	R.S.D. (%)
1.00	1.00	1.8	1.00	4.9	1.00	3.6
5.00	5.00	3.4	4.99	5.0	5.00	2.4
15.00	15.00	2.3	14.99	2.2	15.00	0.6

TABLE II

REPEATABILITY DATA FOR DETERMINATION OF PIROXICAM USING THE PROPOSED METHOD

Theoretical concentration (μg/ml)	Piroxicam found (n = 12)		
	Mean (μg/ml)	S.D. (μg/ml)	R.S.D. (%)
1.00	1.00	0.04	4.1
5.00	5.00	0.19	3.8
15.00	15.00	0.24	1.6

accuracy of the method in terms of recovery was excellent at all tested concentrations. Each measurement was taken as the average of six determinations.

Precision

The precision of the developed method was investigated in terms of inter-day (reproducibility) and intra-day (repeatability) variations. The same three quality control standards were used as described under *Accuracy*.

Reproducibility. Table I shows the results of the precision study in terms of coefficient of variation. Using the proposed extraction and separation scheme, the method gives a precision better than 5% in the concentration range 1–15 μg/ml.

Repeatability. According to the results presented in Table II, the variations in the response of the measuring system when replicate measurements were made on the same samples (quality control standards of 1, 5 and 15 μg/ml) on the same day and under the same conditions did not exceed 5.0%. The results for each concentration are an average of twelve measurements.

Sensitivity

Based on a 20-μl sample volume, the limit of determination (coefficient of variation < 10% on five replicate injections) and the limit of detection (signal-to-noise ratio of 2) of the proposed method were 0.2 and 0.1 μg/ml, respectively.

Application

The method has been successfully applied to the investigation of the steady-state pharmacokinetics of an orally administered 20-mg piroxicam tablet. Fully automated column-switching by PC-controlled software in combination with the direct analysis of plasma made the analysis of up to forty sample vials (two replicate injections from each vial), including the calibration and quality control standards, possible before needing to replace the extraction column packing ma-

terial. The total sample output can further be enhanced by careful planning of the analysis routines. As the system operates without analyst attendance, the analysis can be performed overnight. The sensitivity of the proposed method can be further improved by increasing the sample volume and it can therefore be used to analyse plasma containing low amounts of piroxicam (less than 0.2 μg/ml) with acceptable precision and recovery.

REFERENCES

1 N. E. Pitts and R. R. Proctor, in W. M. O'Brien and E. H. Wiseman (Editors), *Piroxicam*, The Royal Society of Medicine, London, Grune and Strafon, New York, 1978.
2 R. N. Brogden, R. C. Heel, T. M. Speight and G. S. Avery, *Drugs*, 28 (1984) 292.
3 F. Lapicque, P. Netter, B. Bannwarth, P. Trechot, P. Gillet H. Lambert and R. J. Royer, *J. Chromatogr.*, 496 (1989) 301.
4 R. B. Gillilan, W. D. Mason and C. J. Fu, *J. Chromatogr.*, 487 (1989) 232.
5 F. D. Boudinot and S. S. Ibrahim, *J. Chromatogr.*, 430 (1988) 424.
6 J. Macek and J. Vácha, *J. Chromatogr.*, 420 (1987) 445.
7 C. J. Richardson, S. G. Ross, K. L. Blocka and R. K. Verbeeck, *J. Chromatogr.*, 382 (1986) 382.
8 M. T. Kelly, M. R. Smyth and D. Dadgar, *Analyst (London)*, 114 (1989) 1377.
9 R. W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in High-Performance Liquid Chromatography (Journal of Chromatography Library*, Vol. 39A), Elsevier, Amsterdam, 1988.