Journal of Chromatography, 530 (1990) 469–473
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5379

Note

Rapid determination of serum oxatomide levels with on-line precolumn solid-phase extraction

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(First received December 4th, 1989; revised manuscript received April 25th, 1990)

Oxatomide is a new, orally active antiallergic and antihistaminic agent [1]. There is little information on the pharmacokinetics of oxatomide in patients, especially in pediatric patients. Thus, a sensitive method for the determination of serum oxatomide levels is required.

Recently, an octadecyldimethylsilyl phase (ODS) packing material coated with denatured plasma proteins ("protein-coated ODS") was developed [2,3], which has no affinity for plasma proteins but still retains the native ODS characteristics with an affinity for small hydrophobic molecules. An inner surface reversed-phase (ISRP) column has also been developed, which is packed with porous silica gel particles. The surface of these particles is coated with 3-glycerylpropylsilyl phase (diol) but the surface of inner pores is coated with ODS. Serum proteins do not interact with an inner ODS chain of the ISRP column because of the small pore size. Thus, the ISRP column is considered to have a similar property to the protein-coated ODS column.

Deproteinization and/or solvent extraction are essential in conventional analyses before biological samples can be applied to a high-performance liquid chromatographic (HPLC) system, but these steps are complicated and time-consuming [4]. A method involving sample filtration and serum injection was introduced for several drug analyses employing these columns with a column-switching technique. The present study was conducted to develop the analytical method for serum oxatomide levels employing the ISRP column with the column-switching technique, and to apply it to clinical studies.

0378-4347/90/$03.50

EXPERIMENTAL

Materials

Oxatomide was generously supplied from Kyowa Hakko Kogyo (Tokyo, Japan). Organic solvents were purchased from Wako (Osaka, Japan) and distilled prior to use. Other chemicals were of reagent grade and purchased from Wako.

Analytical procedure

An ISRP column (L-1180, 30 mm × 4.6 mm I.D., particle size 10 μm, Chemicals Inspection and Testing Institute, Tokyo, Japan) for deproteinization and an ODS column (LiChrospher RP-18, 250 mm × 4.6 mm I.D., particle size 5 μm, Cica-Merck, Tokyo, Japan) for analytical separation were employed in this study.

Two elution solvents used in this study were 0.01 *M* ammonium acetate (eluent A) for deproteinization and 0.01 *M* ammonium acetate–methanol (20:80, v/v) (eluent B) for analytical separation. Fig. 1 shows schematically the HPLC system. After a flow-direction control valve was set to the discard line and the HPLC system (LC-6A, Shimadzu, Kyoto, Japan) was equilibrated with eluent A at a flow-rate of 1.5 ml/min for 3 min, 300-μl aliquots of serum samples filtered through a disk (Millex SR®, pore size 0.5 μm, Millipore, Bedford, MA, U.S.A.) were injected (7125 injector, Rheodyne, Cotati, CA, U.S.A.) into the HPLC system. Eluates containing serum proteins were discarded for 6 min. Then the flow-direction control valve (HPU-3, Gasukuro Kogyo, Tokyo, Japan) was switched to the analytical column, and the eluent was simultaneously changed to eluent B at a flow-rate of 0.8 ml/min at 50°C (CTO-6A column oven, Shimadzu, Kyoto, Japan). Eluates were monitored by a fluorescence detector (RF-530, Shimadzu) set at an excitation wavelength of 280 nm and an emission wavelength of 309 nm.

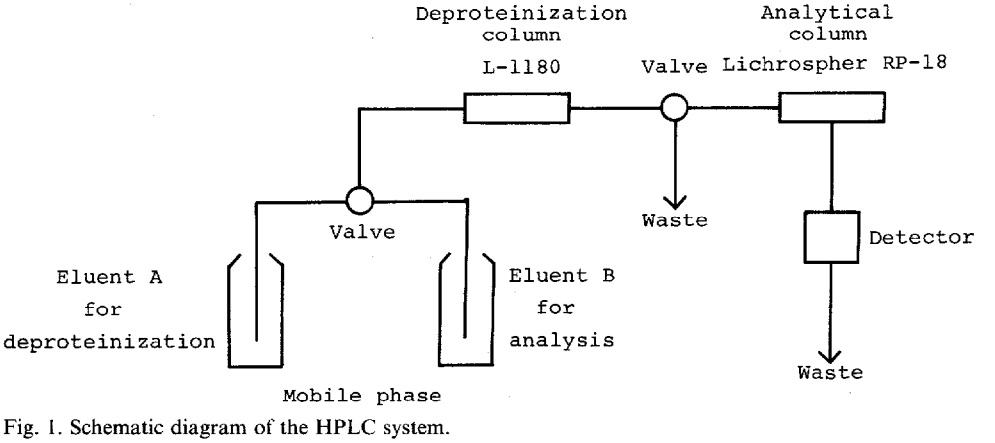

Fig. 1. Schematic diagram of the HPLC system.

Clinical study

Six pediatric patients participated in this study after informed consent was obtained from their guardians. Serum samples were obtained after a single oral dose of 0.5 mg/kg in two patients. Other subjects were given 0.5–1 mg/kg oxatomide in every 12 h. At a steady state, serum samples at the minimum drug levels (C_{ss}^{min}) were collected just prior to the next dosing time. All serum samples were stored at −20°C until analysis.

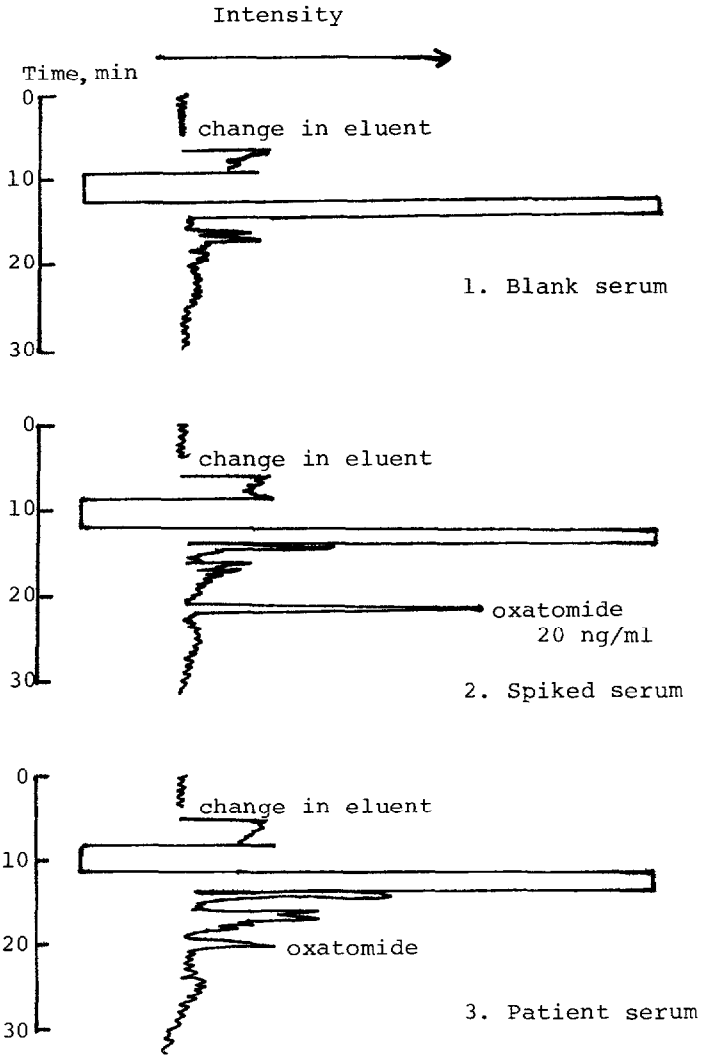

Fig. 2. Typical chromatograms of (1) a blank serum, (2) a blank serum spiked with 20 ng/ml oxatomide and (3) a serum sample from a patient after a single oral administration of 0.5 mg/kg oxatomide.

TABLE I

COEFFICIENTS OF VARIATION OF INTRA- AND INTER-DAY REPRODUCIBILITY OF OXATOMIDE ASSAY ($n = 5$)

Concentration (ng/ml)	Coefficient of variation (%)	
	Intra-day	Inter-day
5	10.5	10.1
20	2.1	10.5
40	2.3	5.5

RESULTS AND DISCUSSION

Typical chromatograms obtained by this method are shown in Fig. 2. Few interfering peaks from endogenous substances were observed. The reproducibility of the method is indicated as the intra- and inter-day coefficients of variation (C.V.) in Table I: all C.V. values for serum oxatomide levels were found to be less than 11%, indicating good reproducibility. The calibration curve was linear up to 50 ng/ml, with a coefficient of correlation of 0.999. The detection limit was found to be 0.5 ng/ml. The mean recovery value of oxatomide in a spiked serum was at least 98%. The total analysis time for a serum sample was found to be less than 30 min.

Michiels *et al.* [4] have reported an HPLC–UV method for plasma oxatomide levels. They tried to clean up plasma drugs by three liquid extraction steps, but their detection limit was reported to be 10 ng/ml for unchanged oxatomide.

Fig. 3 shows time-courses of oxatomide concentrations in serum in two pediatric patients after a single oral administration of 0.5 mg/kg oxatomide. Terminal

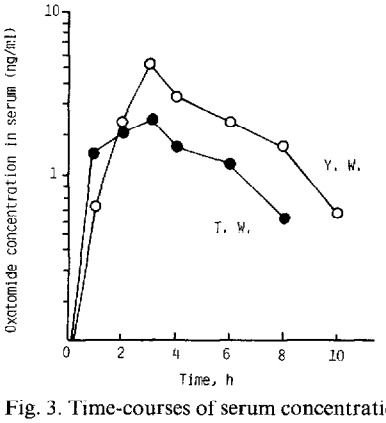

Fig. 3. Time-courses of serum concentrations of oxatomide in two patients after an oral oxatomide dose of 0.5 mg/kg.

elimination half-lives for patients who participated in this study were calculated to be 6.0 and 6.5 h by a log-linear regression analysis. The mean area under the concentration–time curve from 0 to 10 h was 15 ng h/ml. C_{min} levels of oxatomide at the steady state *versus* the daily dose in four pediatric patients were found to be 1–3 ng/ml at the daily dose of 0.5–1 mg/kg. Plasma concentrations of total radioimmunoassayable compounds determined by direct assay of a sample from a dog were already reported to be on average 2.7-fold more after an intravenous administration and, irrespective of the dosing schedule, 5.8-fold after oral administration when compared with values obtained from the assay with plasma extracts [4]. Plasma data obtained with the latter procedure were reported to correlate well with those measured by HPLC [4,5]. Higher values obtained by immunoassay could include concentrations of metabolites of oxatomide, which may show cross-reactivity to the antibody to oxatomide.

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