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Short Communication

High-performance liquid chromatographic method for the analysis of Oltipraz in human serum and urine

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ABSTRACT

A rapid, sensitive procedure for the analysis of Oltipraz in serum and urine using high-performance liquid chromatography was developed. The proposed method illustrates recovery of Oltipraz from biological fluids was greater than 80%. Detection and separation of Oltipraz required as little as 1 ml of serum or urine. Oltipraz was detectable when 2 ng or more of drug was present in 1 ml of serum or urine; the method is highly reproducible when 5 ng/ml or more Oltipraz is present in the biological fluid.

INTRODUCTION

Olitpraz, 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (Fig. 1), is a highly lipid-soluble compound synthesized in 1977 by Rhone-Poulenc Industries [1]. Oltipraz has significant antischistosomal activity in a variety of animals and humans [2]. Recently it has been reported that Oltipraz possesses strong anticarcinogenic properties *in vitro* and *in vivo* [3]. Gas chromatography has been used for the determination of Oltipraz in plasma and urine [4,5]. High-performance liquid chromatographic (HPLC) methods have also been proposed [6,7], however, these studies were done using high doses of Oltipraz (25–35 mg/kg body weight), and the sensitivity of the method and the influence of different experimental conditions were not reported.

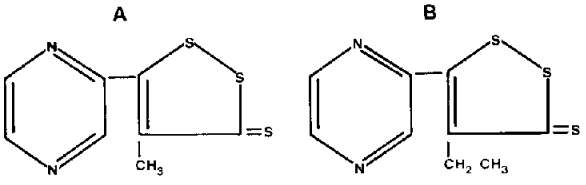

Fig. 1. Structures of (A) Oltipraz, 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione, and (B) the internal standard, 4-ethyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione.

Since low doses (1–5 mg/kg) of Oltipraz may be used as a cancer chemopreventive agent it is necessary to determine the sensitivity of the HPLC method under different experimental conditions [3]. In this communication we describe a method for the analysis of Oltipraz in serum and urine which is sensitive, accurate and reproducible.

EXPERIMENTAL

High-performance liquid chromatography

The chromatographic system employed was a Beckman 331 QC isocratic system with a 313-nm ultraviolet wavelength filter and a 20-μl analytical flow cell. The Beckman 110B solvent-delivery module kept the flow-rate at 1.5 ml/min. The ex-

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tracted samples were injected onto the column through the valve needle port with a 50-μl loop attached. The column used was a reversed-phase Vydac C_{18}, 25 cm × 4.6 cm I.D., 5 μm particle size (Vydac, Hesperia, CA, USA). The total run time for each sample was on average 10 min.

The mobile phase was methanol–0.5 *M* ammonium acetate (6:4, v/v).

Standards and quality control

Aliquots of 1 ml of serum were spiked with 80 ng of internal standard stock solution [4-ethyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (Fig. 1)] and varying amounts of Oltipraz in preparing a standard calibration curve (Figs. 2 and 3). A 1-mg amount of Oltipraz (stock solution) was added to 250 ml of methanol and this was stored at 4°C. Internal standard (2 mg) was added to 250 ml of HPLC-grade methanol and this was also stored at 4°C. These stock solutions were evaluated every week to ensure accuracy. Quality control samples were prepared by adding 40 μl of Oltipaz (from stock solution) to 1 ml of serum and frozen at −80°C. When utilized, the samples were thawed and spiked with 80 ng of internal standard.

Method of extraction

Serum (1 ml) containing varying amounts of Oltipraz was spiked with 80 ng of internal standard. Heptane (3 ml) was added to each milliliter of serum and mixed on a shaker for 1 min. The samples were centrifuged for 15 min at 1500 *g*. With a glass pipette, the heptane (upper layer) was removed and placed into a glass tube with a conical bottom. Heptane (3 ml) was added to the same serum sample and the process was repeated once more. The volume of heptane collected in the conical-bottom tubes after two extractions was 5.5 ml. The heptane was then evaporated to dryness under nitrogen. Immediately prior to injection, 100 μl of methanol were added to the dried sample and shaken for 30 s followed by injection of 70 μl into the loop (50 μl capacity) on the HPLC instrument.

RESULTS AND DISCUSSION

Typical chromatograms from human serum and urine are presented in Figs. 4 and 5. No attempt was made to identify the metabolites of Oltipraz. It has been reported, using rabbit serum

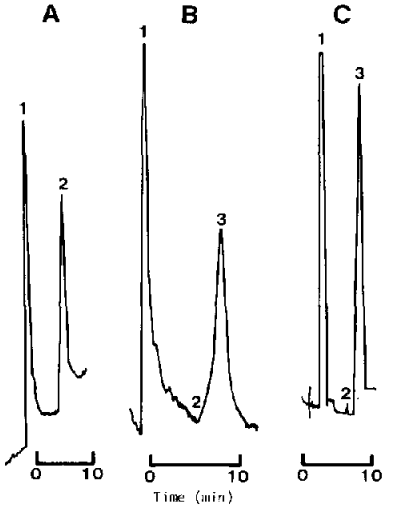

Fig. 2. Chromatograms (A) blank serum (peaks: 1 = solvent front; 2 = internal standard), (B) 2 ng/ml-spiked serum 2 (peaks: 1 = solvent front; 2 = Oltipraz, 3 = internal standard) and (C) 5 ng/ml-spiked serum (peaks: 1 = solvent front; 2 = Oltipraz; 3 = internal standard).

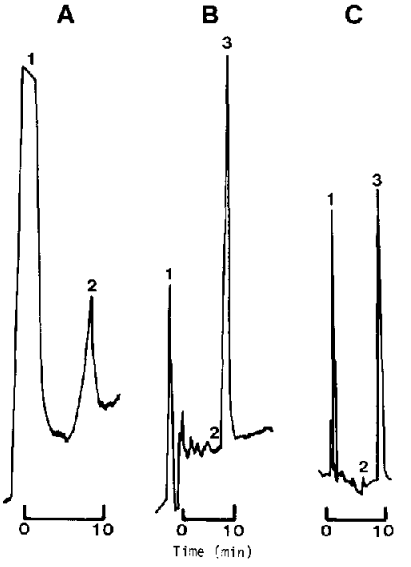

Fig. 3. Chromatograms of (A) blank urine (peaks 1 = solvent front; 2 = internal standard), (B) 2 mg/ml-spiked urine (peaks: 1 = solvent front; 2 = Oltipraz; 3 = internal standard) and (C) 5 mg/ml-spiked urine (peaks: 1 = solvent front; 2 = Oltipraz; 3 = internal standard).

and thin-layer chromatography, that fifteen metabolites could be identified [8].

Oltipraz was detected 1.5 h following ingestion of a single dose (2 mg/kg) by a healthy human volunteer. The retention time was 6 min for Oltipraz while the internal standard was recorded at 8 min. After 24 h, no Oltipraz was detected in the serum (Fig. 4). In a chronic study, a loading dose of Oltipraz (2 mg/kg) was administered at 0 and 6 h and then daily for five or ten days (Fig. 5). A steady state of Oltipraz was detected in the serum daily during the five- or ten-day period of drug ingestion, plus one day after the drug was discontinued.

Oltipraz, administered as a single dose at 2.0 mg/kg, was detected in the urine 3 h after ingestion but not after 24 h (Fig. 5). The chronic patients who received the drug (2 mg/kg) twice on day 1 and then daily for five or ten days, had detectable drug concentrations in their urine until the sixth and eleventh day, respectively (Fig. 4). In urine, the retention time was 5 min for the Oltipraz and 8 min for the internal standard.

Standard calibration mixtures of Oltipraz stock solution ranging from 4 to 120 ng/ml were injected after every three or four samples in order to ensure the consistency of the detector response. Sample concentrations were calculated using peak height and standard calibration curve. The curve was composed by linear regression. The results are presented in Table I.

Five inter- and intra-day evaluations were performed on samples spiked with 40 ng of Oltipraz. For the five inter-day studies the mean was 40 ng with a standard deviation of 1.3. The coefficient of variation was 3%. For the five intra-day studies the mean was 38 ng with a standard deviation of 1.2. The coefficient of variation was 3%. Recovery of spiked samples was 84%.

The proposed HPLC technique for measuring

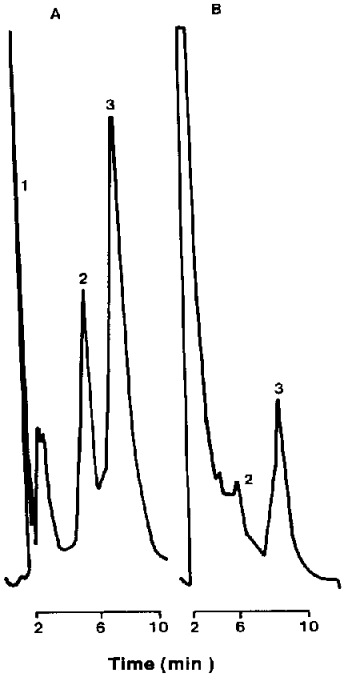

Fig. 4. Chromatograms of serum and urine from a normal subject 6 h after ingestion of Oltipraz, 2 mg/kg body weight. (A) Serum (22 ng/ml); (B) urine (5 ng/ml). Peaks: 1 = solvent front; 2 = Oltipraz; 3 = internal standard. Attention = 0.002.

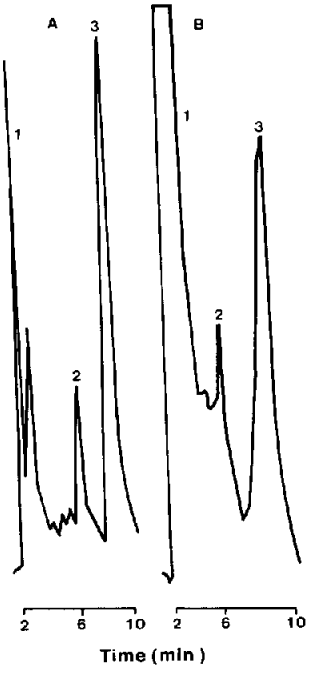

Fig. 5. Chromatograms of serum and urine from a normal subject (same as in Fig. 4) 24 h after ingestion of Oltipraz, 2 mg/kg body weight. (A) Serum (11 ng/ml); (B) urine (10 ng/ml). Peaks: 1 = solvent front; 2 = Oltipraz; 3 = internal standard. Attenuation = 0.002.

TABLE I

CALIBRATION CURVE DATA

Amount of Oltipraz added (ng)	Amount found[a] (ng)	Precision (S.D.)
4	3.9	0.56
10	9.4	0.76
20	17.4	0.75
40	37.2	0.63

[a] Five runs were performed for each concentration.

Oltipraz in human serum is both rapid, accurate and reproducible, but more importantly, it is fifty times more sensitive than our previously published method [7]. In addition, the ability to detect Oltipraz in the urine is important and will be used as a tool for monitoring compliance during chemoprevention trials.

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