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Note

Analysis of olivetol in rabbit serum by high-performance liquid chromatography*

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Phenolic compounds have been found to be effective inhibitors of prostaglandin cyclooxygenase[1]. Olivetol is a phenolic compound (3,5-dihydroxy-*n*-pentylbenzene) found in substantial quantities in pyrolysis products of cannabidiol and could contribute to harmful effects produced by smoking marijuana[2]. Analysis of cannabinoids by gas chromatography has been shown to produce detectable quantities of olivetol as a result of pyrolytic reactions in the injection port[3]. During the course of a study of the effect of olivetol on uterine blood flow in rabbits it was necessary to develop a method for analysis of olivetol in serum. Gas chromatography did not prove to be useful due to tailing peaks when olivetol was not derivatized and multiple products when it was derivatized (pentafluoropropionate). Therefore, the following straightforward and sensitive high-performance liquid chromatographic (HPLC) method was developed.

EXPERIMENTAL

HPLC was performed on a reversed-phase μBondapak C_{18} column (20 cm × 3.9 mm I.D., 10 μm particle size, Waters Assoc., Milford, MA, U.S.A.) with acetonitrile–water (40:60) as the mobile phase. The flow-rate was 2.0 ml/min at a pressure of 2000 p.s.i. Olivetol was detected at a wavelength of 280 nm (Waters 440 UV detector). Peak areas were calculated by triangulation.

Serum samples (1 ml) were extracted three times with 5 ml of diethyl ether. The combined ether extracts were concentrated to about 1 ml, the sides of the tube were washed down with absolute ethanol, and this was concentrated to near dryness again. Acetonitrile was then used to wash the sides of the tubes several times to replace the ethanol with acetonitrile. This was concentrated to near dryness and 50 μl of acetonitrile were added. Any insoluble material was removed by centrifugation. The total quantity of solution (<50 μl) was injected into the HPLC column.

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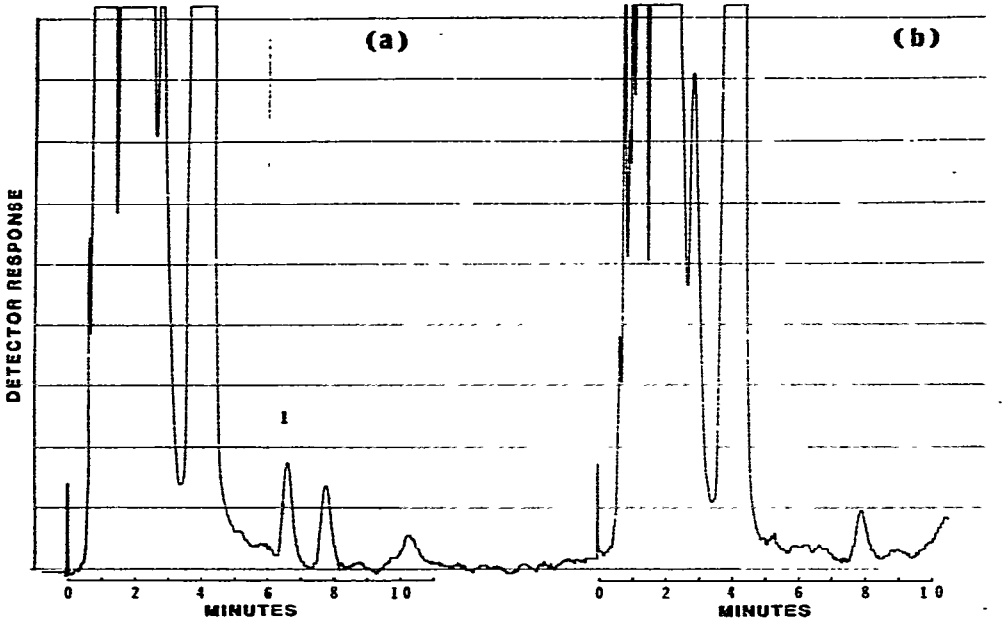

Fig. 1. Chromatogram of rabbit serum extract at maximum sensitivity (0–0.005 a.u.f.s.). See Experimental section for HPLC details. (a). Extract from 1 ml of serum spiked with 100 ng of olivetol (peak 1). (b). Serum blank.

RESULTS AND DISCUSSION

Gas chromatographic analysis of olivetol required derivatization (pentafluoropropionate) for optimum peak shape. However, incomplete derivatization was found to be a problem as evidenced by a broad second peak that appeared when using OV-210 as the stationary phase or a sharp second peak when using SE-30. Also, interferences appeared when serum extracts were derivatized.

Reversed-phase HPLC (μBondapak C_{18}) of olivetol using acetonitrile–water (40:60) as mobile phase gave a symmetrical peak eluting with a retention time of 6.7 min (flow 2.0 ml/min). A 50-mm guard column packed with a reversed-phase packing (Supelco LC-8) was placed in front of the analytical column.

The absorbance maximum of olivetol is 278 nm and the HPLC procedure proved to be very sensitive when monitoring with a 280 nm filter. The standard curve was linear down to 20 ng with a correlation coefficient of 0.998 (each point a mean of 3 determinations). The standards did not deviate from linearity until 1000 ng were injected giving a region of linearity from 20 ng to 800 ng.

No adequate internal standard was readily available. A recovery study was conducted by spiking eight 1-ml serum samples with 500 ng of olivetol standard (Polysciences, Warrington, PA, U.S.A.). This quantity was chosen because the biological sample concentrations were in this range. The mean recovery was 82% with a standard deviation of 6.3% and a range of 72% to 92%.

The greatest source of variability was the dissolution of the extracted olivetol in acetonitrile for injection on the HPLC column. Not all of the components of the

extract were readily soluble in acetonitrile so it was necessary to maintain the solutes in solution by a gradual transfer of the solvent from ether to ethanol to acetonitrile.

The serum blank was exceptionally free of interfering peaks even at maximum sensitivity (Fig. 1). A gradual buildup of highly lipophilic materials necessitated a daily cleaning of the column with acetonitrile as well as periodic cleaning of the guard column.

This procedure proved to be highly sensitive and free of interferences when applied to biological samples. It has been used to satisfactorily quantitate olivetol in a large number of rabbit serum samples.

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