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Note

Gas chromatographic method for the estimation of acetone and its metabolites in biological samples

J. PEINADO, F.J. LÓPEZ-SORIANO and J.M. ARGILÉS*

Fisiologia General, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona (Spain)

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Since Thin and Robertson [1] reported a colorimetric method for the determination of acetone in blood, several publications have appeared describing improved procedures for its measurement based on gas chromatographic (GC) analysis [2], often combined with the assay of other ketones [3,4].

In mammals, acetone is generated from acetoacetate [5] and has normally been regarded as an unmetabolized waste product excreted via the lungs and kidneys. However, studies with ^{14}C-labelled acetone in lactating cows [6], rats [7], and guinea pigs [8] and humans [9] have revealed that acetone can be further metabolized via two main pathways in which hydroxyacetone (acetol), methylglyoxal (2-ketopropanal), 1,2-propanediol and 2,3-butanediol are involved as intermediates. Both pathways are considered gluconeogenic [7,10] since they lead to the formation of pyruvate.

To date, no GC method has been reported for the combined determination of acetone and its metabolically related compounds. This lack of GC is due both to the difficulties of chromatographing the relatively small concentrations of these compounds and the very limited information concerning acetone metabolism in mammals.

We report here a simple, rapid and reliable GC method, which is applied to the measurement of these metabolites in plasma and liver of rats intravenously injected with acetone.

EXPERIMENTAL

Reagents

Acetone (Carlo-Erba, Milan, Italy), acetol (Fluka, Buchs, Switzerland), methylglyoxal (Sigma, St. Louis, MO, U.S.A.), 1,2-propanediol (Carlo-Erba),

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2,3-butanediol (Carlo-Erba) and 2-ethoxyethanol (oxitol) (Probus, Barcelona) were used. All the other chemicals were of analytical grade.

Animals and preparation of samples

Adult female Wistar rats weighing 224 ± 12.6 g were used as a source of blood and liver in the experiments. They were fed a stock chow diet and water ad libitum and maintained in a room where the temperature ($22 \pm 2°C$) and light cycles were controlled. They were given an intravenous injection of a 3% (w/v) acetone solution (100 mg/kg body weight) in physiological saline (0.9% sodium chloride) through one of the tail veins. The animals were sacrificed 240 min after the injection by decapitation and their blood and liver immediately removed. Plasma was obtained after centrifugation for 30 min at 1500 *g* in a refrigerated centrifuge. Liver tissue was frozen by means of liquid nitrogen and pulverized in a cold mortar. Then 300 mg of tissue powder were placed in a test-tube containing 1 ml of cold 0.6 *M* perchloric acid. The contents of the tube were homogenized and then centrifuged for 30 min at 1500 *g* at 4°C. The clear supernatants were transferred to another tube and neutralized with a saturated solution of potassium hydrogen carbonate. Plasma samples were deproteinized using the same procedure, and 200 μl of plasma were mixed with 500 μl of cold 0.6 *M* perchloric acid. After mixing, the samples were centrifuged for 30 min, the clear supernatants being then neutralized with potassium hydrogen carbonate. Deproteinized liver tissue and plasma were kept frozen at $-40°C$, prior to analysis.

Chromatographic methodology

The gas chromatograph was a Perkin-Elmer (Norwalk, CT, U.S.A.), Model 3B, equipped with a flame ionization detector and a Sigma 15 integrator and recorder.

For the separation of acetone, 1,2-propanediol and 2,3-butanediol, the column used was a Carbopack C 80–100 mesh, 0.8% THEED (Supelco, Bellefonte, PA, U.S.A.) packed into a stainless-steel column (1 m$\times$3.17 mm I.D.). Instrument settings were: oven, injector and detector temperatures, 115°C; hydrogen flow-rate, 20 ml/min; air flow-rate, 300 ml/min; carrier gas (nitrogen) flow-rate, 60 ml/min. Oxitol was used as an internal standard and was added to the samples before injection. The retention times of acetone, 1,2-propanediol, 2,3-butanediol and oxitol were 0.24, 7.34, 9.64 and 1.96 min, respectively. The sample volume was 5 μl.

For the separation of acetol and methylglyoxal, the column used was a Porapack QS 80–100 mesh (Supelco), coated with 3% Carbowax 20M packed into a 2-m glass column. Instrument settings were: oven temperature, 170°C; injector and detector temperatures, 200°C; hydrogen flow-rate, 20 ml/min; air flow-rate, 300 ml/min; carrier gas (nitrogen) flow-rate, 60 ml/min. No internal standards were used for the quantification of the samples. The retention times of methylglyoxal and acetol were 2.41 and 11.57 min, respectively. The sample volume was 5 μl.

Calculations

Quantification was performed from the plot of the acetone/oxitol, 1,2-propanediol/oxitol and 2,3-butanediol/oxitol concentrations, respectively. For the mea-

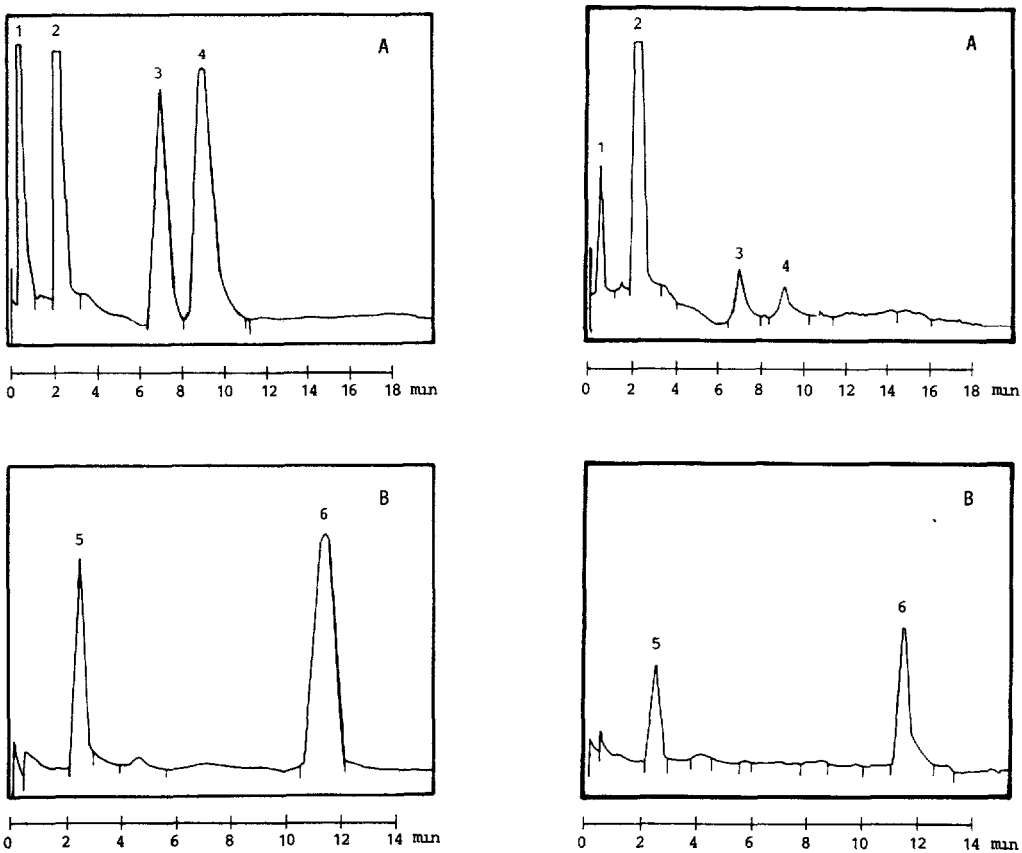

Fig. 1. Typical GC separations of reference compounds on two columns. (A) Carbopack C 80–100 mesh, 0.8% THEED; peaks: 1 = acetone, 0.45 m*M*; 2 = oxitol, 0.45 m*M*; 3 = 1,2-propanediol, 0.45 m*M*; 4 = 2,3-butanediol, 0.45 m*M*. (B) Porapack QS 80–100 mesh, 3% Carbowax 20M; peaks: 5 = methylglyoxal, 0.45 m*M*; 6 = acetol, 0.90 m*M*.

Fig. 2. Typical GC separations of acetone and its metabolites in plasma. (A) Carbopack C 80–100 mesh, 0.8% THEED; peaks: 1 = acetone, 197.6 μM; 2 = oxitol, 0.45 m*M*; 3 = 1,2-propanediol, 38.4 μM; 4 = 2,3-butanediol, 28.3 μM. (B) Porapack QS 80–100 mesh, 3% Carbowax 20M; peaks: 5 = methylglyoxal, 850.0 μM; 6 = acetol, 773.1 μM.

surement of methylglyoxal and acetol, standard solutions of these compounds in physiological saline were used. The calibration curves were linear over the concentration range of interest. In all cases, the quantification was performed using peak areas.

RESULTS AND DISCUSSION

Figs. 1 and 2 show typical chromatograms obtained for the separation of the different metabolites on both the Carbopack and the Poropack columns. The resolution of the peaks was baseline or near baseline. As can be seen, there is no solvent peak because an aqueous solution was used. Thus, a blank sample with

TABLE I

ACETONE, ACETOL, METHYLGLYOXAL, 1,2-PROPANEDIOL AND 2,3-BUTANEDIOL LEVELS IN PLASMA AND LIVER TISSUE AFTER INTRAVENOUS INJECTION OF ACETONE TO FEMALE RATS

Values are expressed as mean ± S.D. The number of animals used is indicated in parentheses.

Compound	Plasma (μM)	Liver (μmol/kg fresh tissue)
Acetone	199.6 ± 24.2 (2)	115.5 ± 98.6 (3)
Acetol	759.3 ± 85.1 (4)	3912.0 ± 367.5 (5)
Methylglyoxal	867.3 ± 32.0 (4)	1255.0 ± 100.0 (4)
1,2-Propanediol	40.8 ± 2.7 (3)	25.9 ± 12.6 (3)
2,3-Butanediol	30.2 ± 10.4 (4)	1.5 ± 0.0 (1)

no internal standard gives a flat baseline. All these volatile compounds could be accounted for in either deproteinized plasma or liver tissue. Detector responses were linear for these compounds from zero up to concentrations in the physiological range ($y = 1.88 + 24.71x$, $r = 0.9999$ for methylglyoxal; $y = 8.19 + 60.63x$, $r = 0.9973$ for acetol). The linearity of the detector response for the internal standard used was also satisfactory ($y = 0.70 + 87.29x$, $r = 0.9999$). The methodology is sensitive, easily capable of quantifying peaks representing concentrations of less than 10 μM. The individual detection limits (nmol) for the different compounds were 0.023 for acetone, 0.025 for 1,2-propanediol, 0.023 for 2,3-butanediol, 0.15 for methylglyoxal and 0.14 for acetol (signal-to-noise ratio = 2). The method has also been applied to measure physiological concentrations of these compounds in chronic ethanol-treated animals [11].

Although there has been some work done on the separation and estimation of some of these compounds by either GC [12] or liquid chromatography [13], the major advantage of this methodology is that it allows for the separation of both acetone and its metabolites, giving rapid and sensitive results in a relatively short time. On the other hand, interest in acetone metabolism has been increasing in the past few years. In lactating cows, the incorporation of ^{14}C atoms from [2-^{14}C]acetone into glucose has been demonstrated [7], this transformation having also been described in mice [14]. More recently, some authors [8,11] have described the different pathways by which acetone can be a gluconeogenic precursor. These pathways involve all the compounds estimated by the GC methodology proposed here. In addition, 2,3-butanediol is related to ethanol metabolism, and it has been shown that ethanol increases serum acetone in rats [15]. Table I shows the concentrations of the related compounds in plasma and liver tissue of rats injected intravenously with a single dose of acetone. These results illustrate the methodology proposed.

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