# A simple, rapid method for measurement of acetate in tissue and serum

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**Summary** A simple and rapid method is described for determining the free acetate concentration in liver and serum. After extraction, the acetate is converted to its benzyl ester by thermal degradation of its benzyldimethylphenylammonium salt in the vaporizer of a gas chromatograph. Good quantitation is achieved in the range of 0.033–2.5 μmoles of acetate per gram of liver or per milliliter of serum.

**Supplementary key words** liver · gas–liquid chromatography · benzyl esters · benzyldimethylphenylammonium hydroxide · quaternary ammonium hydroxide

Several procedures have been developed to measure acetate in tissue and serum (1–7). All of these methods are tedious or time-consuming, or they require partial isolation of enzymes or special apparatus. This study was initiated to develop a simple and rapid method for determination of acetate concentrations in biological materials.

Using the vaporizer of a gas–liquid chromatograph as a reactor, tetramethylammonium hydroxide will methylate carboxylic acids by the thermal elimination of trimethylamine from the quaternary ammonium salts (8). Trimethylphenylammonium hydroxide has also been used successfully to methylate long-chain fatty acids, with the advantage that the reaction occurs at a lower temperature (9).

For short-chain carboxylic acids such as acetate, benzyl esters have been reported to give good chromatographic separation on EGSS-X (10).

Based on these reports, we have developed a simple method for extraction and benzylation of acetate from serum and from tissue homogenates using benzyldimethylphenylammonium hydroxide (BDMPH).

*Materials.* BDMPH (0.1 M) was prepared by vigorously agitating benzyldimethylphenylammonium chloride (Eastman Kodak) on a Vortex mixer in the presence of excess silver oxide (Fisher Scientific) and distilled water. The precipitate was removed by centrifugation, and the supernatant fluid was tested for chloride with 0.1 M silver nitrate in 6 M nitric acid. If a positive halide test was observed, additional silver oxide was added and the procedure was repeated until no chloride could be detected.

*Tissue extraction.* The tissues selected for analysis were rat liver and human serum. Liver was quick-frozen in liquid nitrogen and pulverized with a mortar and pestle. The pulverized liver, 0.5–1.5 g, was homogenized in 3 ml of distilled water containing 0.405 μmole of propionate as an internal standard. The homogenate was acidified with 0.3 ml of 5 M perchloric acid, mixed, and allowed to stand at room temperature for 10 min. The sample was centrifuged at 1500 *g* for 10 min, and the supernatant fluid was decanted and recentrifuged for an additional 5 min to remove any remaining cellular debris. Tissue homogenization directly in 0.5 M perchloric acid was found to yield results identical with those observed after homogenization in distilled water followed by acidification. The former technique was used because it eliminated the problem of tissue protein precipitation during homogenization. For serum extraction, 0.135 μmole of propionate and 0.1 ml of 5 M perchloric acid were added to 1 ml of serum. The contents were mixed and processed in a manner identical with liver.

1 ml of the final supernatant fluid was added to a 15-ml glass-stoppered centrifuge tube containing 5 ml of toluene and 1 g of anhydrous sodium sulfate. The tube was shaken vigorously by hand for 1 min and centrifuged at 600 *g* for 1 min. The toluene phase was transferred to a 5-ml glass-stoppered conical centrifuge tube (Corning no. 8061). Care was taken to avoid transferring any of the aqueous phase. 10 μl of the aqueous BDMPH was then added. The mixture was shaken and centrifuged as above. Aliquots of the resulting lower phase were now ready for withdrawal with a 10-μl Hamilton syringe prewetted with water for chromatographic analysis.

*Instrumentation.* Gas–liquid chromatography was performed on an F & M high efficiency chromatograph model 402 with 6 ft × 0.25 inch glass U columns. The columns were packed with pretested 10% EGSS-X on Gas-Chrom P, 100–120 mesh (Applied Science, lot SP-1076). The injection port was maintained at 260°C and the column at 130°C. The carrier gas was nitrogen at 35 ml/min. The flame ionization detector was operated at 220°C.

*Quantitation.* Sodium propionate (Fisher) was used as an internal standard because, using this technique, no endogenous propionic acid could be detected in either liver or serum. External standards of sodium acetate (Fisher) were prepared in various concentrations from 0.033 to 2.50 μmoles of acetate/0.135 μmole of propionate. The compounds were identified by comparing retention times with known benzyl esters (Eastman) and by mass spectrometry. The areas under the benzyl acetate and benzyl propionate peaks were determined by the method of Ackman and Bur-

Abbreviations: BDMPH, benzyldimethylphenylammonium hydroxide.

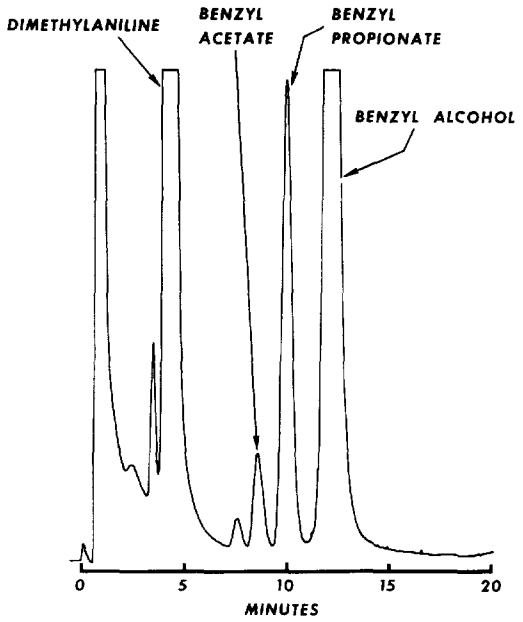


**Fig. 1.** Gas-liquid chromatogram of 1 ml of serum plus 10 μg of propionate on EGSS-X after extraction with toluene and BDMPH.

gher (11). **Fig. 1** is a typical chromatogram. Quantitation of acetate was determined from the plot of the acetate:propionate ratio vs. the acetate concentration.

*Results and discussion.* Using this method, the total time from tissue homogenization to completion of the chromatography is less than an hour. Neither acetate nor propionate is extracted quantitatively into the toluene. In fact, the extraction of acetate in this step is only about 15%, even with the addition of sodium sulfate. However, because the samples used to prepare the standard curve and the specimens are treated identically, absolute recovery is not necessary, but good reproducibility is essential. Toluene was found to be more suitable for extraction than other solvents tested; these included hexane, petroleum ether, and diethyl ether. The extraction of the acetic acid from the toluene phase by BDMPH was essentially 100% because additional extractions of the toluene phase showed no acetate or propionate. Base-line resolution was observed for both benzyl acetate and benzyl propionate with retention times of 9.0 and 10.4 min, respectively. Purity of the benzyl acetate peak was established on an LKB-9000 gas chromatograph–mass spectrometer. No fragmentation spectrum was seen other than that for benzyl acetate.

The standard curve, prepared using 0–2.5 μmoles of acetate, yielded a straight line passing through the origin when the peak-area ratio was plotted vs. the acetate concentration. Using this type of standard curve to analyze six different amounts of acetate added in quadruplicate to a liver homogenate yielded apparent recoveries of 100.5 ± 9.6% (mean ± SD). As seen in **Fig. 2,** the recovery of known amounts of acetate added to a tissue homogenate is both linear and quantitative, with a correlation coefficient ($r$) of 0.998.

The precision of the method was measured from nine determinations performed on rat liver and nine determinations on pooled human serum. The acetate concentrations were (mean ± SD) 1.117 ± 0.079 μmoles of acetate/g of liver and 10.8 ± 0.89 μmoles of acetate/100 ml of serum. This agrees well with values found by Lester (5).

The advantages of this assay system are that it is simple, quick, and practical for the determination of free acetate in liver and serum using conventional gas–liquid chromatographic instrumentation and easily available reagents.

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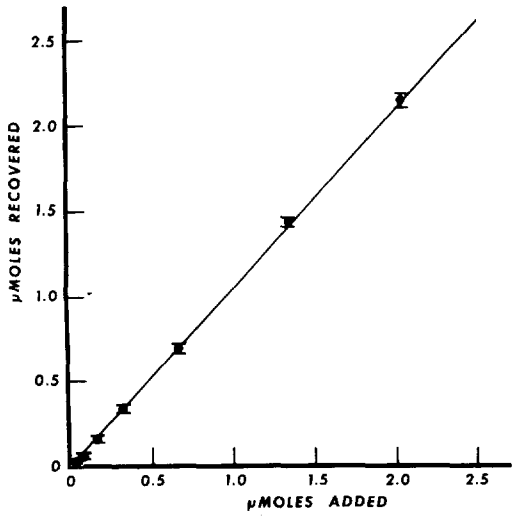


**Fig. 2.** Recovery of acetate from liver homogenate. Each point represents the mean ± SD of four determinations.

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