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DETERMINATION OF SODIUM CAPRYLATE IN PLASMA VOLUME EXPANDERS BY GAS CHROMATOGRAPHY

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SUMMARY

A quantitative gas chromatographic method for the determination of the sodium (caprylate) octanoate, antimicrobial in the plasma volume expanders hydroxyethyl starch and human serum albumin, has been developed. The sodium caprylate and the internal standard were converted to pentafluorobenzyl derivatives. The reaction mixture was extracted with methylene chloride and chromatographed on a 1.8-m OV-17 column at 170°C with flame ionization detection. The method is linear over the concentration range studied (40–700 μg/ml). The method is precise (coefficient of variation <2%).

INTRODUCTION

One of the commercially available plasma volume expanders, human serum albumin, contains the antimicrobial sodium caprylate, which is chemically known as sodium octanoate. Methods for the determination of specific fatty acids and their salts, such as sodium caprylate, in a wide range of media have been the subject of recent review articles[1–4].

Extractive alkylation is a convenient method for derivatization of acids. It has the advantage of removing the organic compound of interest from the water-soluble components of the sample. This type of derivatization technique has been described earlier for dodecanoic and hexadecanoic acids[5]. These acids were alkylated with pentafluorobenzyl bromide in the presence of tetrabutylammonium hydrogen sulfate to form the corresponding ester and extracted into methylene chloride for subsequent analysis by gas chromatography with electron capture detection in a single step.

The method used in this laboratory for the gas chromatographic determination of sodium caprylate in the plasma volume expanders hydroxyethyl starch and human serum albumin by gas chromatography with flame ionization detection is described. The effect of reagent concentration on reaction time and conditions are discussed.

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EXPERIMENTAL

Apparatus

A Hewlett-Packard (Avondale, PA, U.S.A.) Model 5830A gas chromatograph equipped with a flame ionization detector was used for this study. A glass column (1.8 m × 4 mm I.D.) was packed with 3% OV-17 on 100–120 mesh Chromasorb W-HP obtained from Alltech (Deerfield, IL, U.S.A.). Analyses were carried out isothermally at 170°C, with injection port and detector temperatures at 195° and 300°C, respectively. A Hewlett-Packard Model 5985 mass spectrometer with an ionization energy of 70 eV was used to verify the structure of the methyl octanoate derivative.

Reagents

Pentafluorobenzyl bromide and tetrabutylammonium sulfate were obtained from Pierce (Rockford, IL, U.S.A.). Caprylic (octanoic) acid was obtained from Aldrich (Milwaukee, WI, U.S.A.) and 2-phenylpropionic acid from Pfaltz and Bauer (Stamford, CT, U.S.A.). Hexanoic, heptanoic, nonanoic and dodecanoic acids were obtained from Alltech. All organic solvents were analytical grade obtained from Burdeck and Jackson (Muskegaon, MI, U.S.A.). All reagents were used as received. Serum albumin containing sodium octanoate (Pierce), and 10% human serum albumin (Cutter Labs., Berkeley, CA, U.S.A.) and hydroxyethyl starch (McGaw Labs., Irvine, CA, U.S.A.) were also used as received.

Solutions of 20% hydroxyethyl starch were prepared by dissolving 20 g of hydroxyethyl starch, 500 mg of sodium chloride and 330 mg of sodium caprylate in water to yield a final volume of 100 ml.

Sample preparation

A 5 ml portion of each of the samples, hydroxyethyl starch with and without sodium caprylate and human serum albumin with and without sodium caprylate, were pipetted into individual 50-ml volumetric flasks and diluted to volume with water. The final theoretical concentration of sodium caprylate for commercial human serum albumin and the hydroxyethyl starch samples were 332 μg/ml ($2 \cdot 10^{-3}$ *M*) and 66 μg/ml ($4 \cdot 10^{-4}$ *M*), respectively.

Aliquots of 1 ml of samples or standards, 1 ml of 0.8 mg/ml 2-phenylpropionic acid in 0.1 *M* sodium hydroxide and 0.5 ml of 0.2 *M* tetrabutylammonium hydrogen sulfate in 0.2 *M* sodium hydroxide were added to 2 ml of 0.066 *M* pentafluorobenzyl bromide in methylene chloride. The mixture was shaken for 2 h at room temperature in a mechanical shaker operating at approximately 60 strokes per min to achieve a *g* value of 925. The samples and standards were then centrifuged at 2000 rpm for 5 min. The organic layer was separated from the aqueous layer. A 2-μl aliquot of the organic layer was injected into the gas chromatograph.

Calibration curves were prepared by adding sodium caprylate to aqueous media. Standards were derivatized, extracted and chromatographed simultaneously with samples. Typical calibration curves for hydroxyethyl starch and human serum albumin were in the 100–700 μg/ml range. A least-squares linear regression analysis of the concentration *vs.* peak-area ratio (caprylate ester/2-phenylpropionate ester) was used to calculate the amount of sodium caprylate.

RESULTS AND DISCUSSION

A derivatization experiment with individual C_4–C_{10} acids showed separation of the fatty acid esters from one another and from the internal standard, as shown in Fig. 1. Representative chromatograms of the pentafluorobenzyl derivative of sodium caprylate in hydroxyethyl starch, human serum albumin and distilled water are shown in Fig. 2. The retention times for the derivatized caprylate and 2-phenylpropionate were 4.6 and 7.9 min, respectively. Hence, fatty acid impurities potentially present in sodium caprylate can be separated in hydroxyethyl starch and human serum albumin preparations and do not interfere with the determination.

The internal stadard, 2-phenylpropionic acid, was chosen because its chromatographic retention is significantly different from that of caprylic acid and the other fatty acids (Fig. 1). The perfluorobenzyl bromide reagent is common used to enhance detection with electron capture detectors[5]. It was used for this application to speed reaction with caprylate and other anions[6] and to increase sample volatility.

The optimum molar ratios of sodium caprylate and the internal standard were determined by following the conversion of 2-phenylpropionic and caprylic pentafluorobenzyl esters, as shown in Fig. 3. Two parameters were varied to optimize recovery. The concentration of PFBB ranged from 0.025 to 0.066 *M* and those for

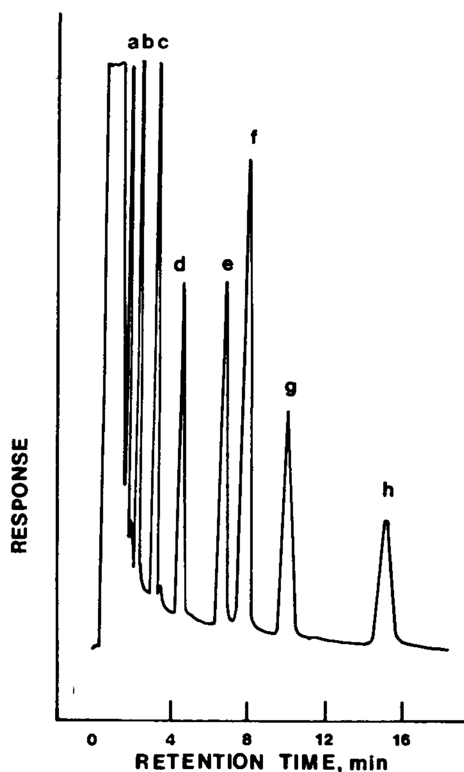
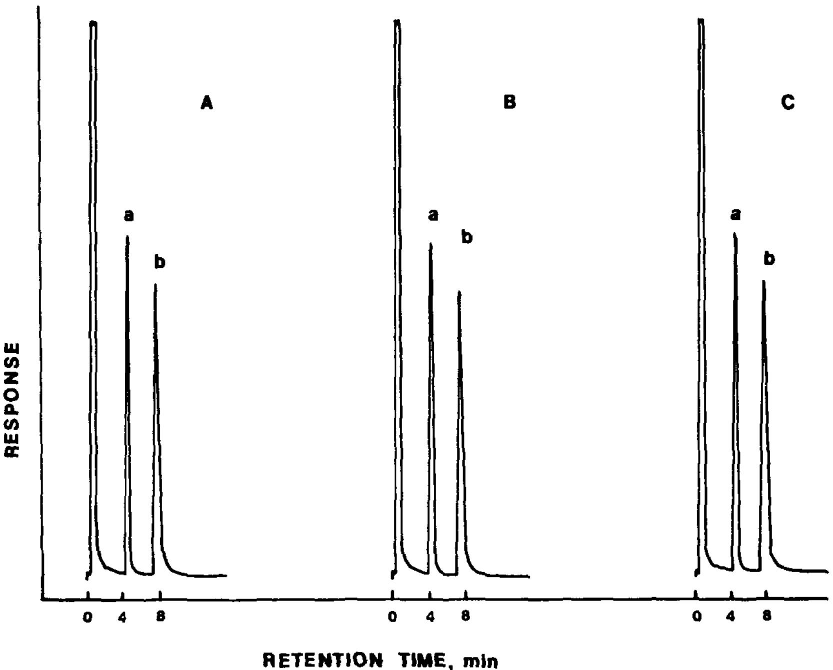


Fig. 1. Separation of C_4–C_{10} fatty acids. Peaks: a = C_4; b = C_5; c = C_6; d = C_7; e = C_8; f = C_9; g = internal standard; h = C_{10}.



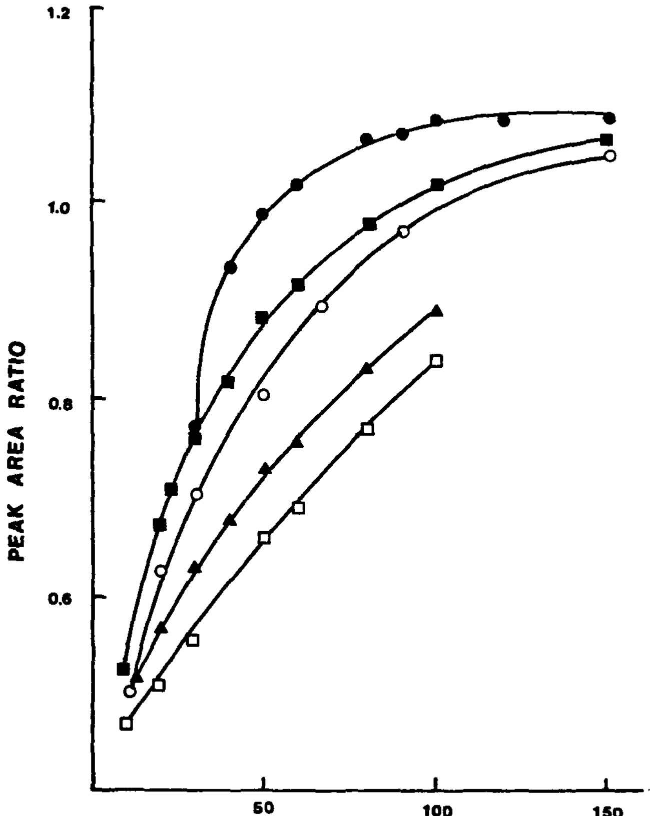


Fig. 2. Chromatograms of sodium caprylate in (A) water, (B) hydroxyethyl starch, (C) albumin. a = Sodium caprylate; b = internal standard.

Fig. 3. The effect of pentafluorobenzyl bromide (PFBB) and tetrabutylammonium hydrogen sulfate (TBA) concentration on the rate and extent of alkylation: (●) 0.2 *M* TBA, 0.066 *M* PFBB; (■) 0.1 *M* TBA, 0.066 *M* PFBB; (○) 0.1 *M* TBA, 0.050 *M* PFBB; (▲) 0.1 *M* TBA, 0.037 *M* PFBB; (□) 0.1 *M* TBA, 0.025 *M* PFBB.

TABLE I

RECOVERY OF SODIUM CAPRYLATE FROM HUMAN SERUM ALBUMIN AND HYDROXYETHYL STARCH SAMPLES

Sample	*Amount of caprylic acid added (µ/ml)*	*Amount of caprylic acid found (µg/ml)*	*Recovery* (%)*	*Relative standard deviation***
Human serum albumin	40	36	90	1.9
	100	101	101	1.9
	200	202	101	1.8
	500	501	100	0.9
	1000	999	99.8	1.0
	0	3414**	102.8	1.0
	1000	4167*	96.5	1.5
	2000	5407*	101.6	0.8
	3000	6524**	103.2	0.6
	4000	7350**	100.4	1.9
Hydroxyethyl starch	0	0	–	–
	40	39	98	1.6
	100	98	101	1.9
	200	200	100	0.9
	400	404	101.2	0.9
	500	513	100.3	1.1
	1000	1003	102.7	0.4
	2000	1975	98.7	1.8
	3000	2939	98.0	0.5
	4000	3930	98.3	0.9
	5000	4926	99.6	0.8

* Average of four determinations.

** Based on amount of sodium capryate added to commercial samples containing 3320 µg/ml sodium octanoate.

TBA from 0.1 to 0.2 *M*. Best results were obtained with 0.066 *M* PFBB and 0.2 *M* TBA. The yield of 2-phenylpropionate and caprylate pentafluorobenzyl esters was constant for reaction times between 100 and 160 min. It was impossible to differentiate between derivatives formed prior to extraction and that formed in the injector[7].

The identity of the esters of caprylic acid and 2-phenylpropionic acid were verified by mass spectrometry. Molecular ions observed at *m/e* 324 and 330 correspond to the molecular weights of the caprylic and 2-phenylpropionic esters, respectively. Both esters contained *m/e* 181 (perfluorobenzyl) as the base peak. These data and further fragmentation confirm the identity of the derivatives.

Hydroxyethyl starch and human serum albumin samples were spiked with varying amounts of sodium caprylate. These samples were assayed in quadruplicate (Table I). The limit of detection was 4 µg/ml. Good quantitative reproducibility was observed at 40 µg/ml.

Linearity was studied with (a) aqueous sodium caprylate standards, (b) hy-

droxyethyl starch samples prepared by standard addition of aliquots of sodium caprylate standard solution, and (c) standard addition of aliquots of sodium caprylate standard solution to commercial human serum albumin samples containing sodium caprylate. The gas chromatographic (GC) system response (ratio of the caprylate peak to the area of the internal standard *vs.* concentration was linear up to the 700 μg/l level. The results of linear regression analysis were: slope = 1.00, intercept −0.030, correlation coefficient = 0.9994 for aqueous standards; slope = 1.00, intercept 0.111, correlation coefficient = 0.998 for spiked hydroxyethyl starch samples; slope = 0.997, intercept, 0.655 and correlation coefficient = 0.9974 for spiked human serum albumin samples.

CONCLUSIONS

The method reported here offers a simple means of determining sodium caprylate in the two widely used plasma volume expanders via derivatization/extraction and subsequent gas chromatographic analysis. Caprylic acid is separated from other straight-chain fatty acids. The method shows 102.8% recovery of theoretical from commercial albumin. Excellent recoveries are exhibited at 3% of theoretical. Furthermore, the high reproducibility of this method is demonstrated by relative standard deviations over two concentration decades of less than 2%.

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