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# QUANTITATIVE GAS—LIQUID CHROMATOGRAPHY OF TAURINE

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## SUMMARY

A method has been developed for the determination of taurine by gas—liquid chromatography. The method involves the conversion of taurine into its N-isobutyloxycarbonyl di-*n*-butylamide derivative and chromatography on a 1.5% OV-17 column. The derivative can be prepared in quantitative yield, having good chromatographic properties. The calibration curve for taurine in the range 5—500 nmol was linear and sufficiently reproducible for quantitative determination. Complex biological material such as urine can be analysed accurately and precisely by this method without prior clean-up of the sample.

## INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is one of the most abundant and ubiquitous free amino acids in the fluids and tissues of animals [1], and much evidence suggests that it plays an important role in the body [2].

The determination of taurine in biological material has been carried out by colorimetric [3—8], fluorometric [9, 10], radiometric [11] and enzymatic [12] assay methods. However, these methods usually require time-consuming pretreatment of the sample [13]. Chromatographic procedures utilizing an amino acid analyzer [13—19] or high-performance liquid chromatographic system [20—24] have also been used for the assay, but when applying them directly to the biological sample there appears to be difficulty in resolving taurine from interfering components [17—19, 23]. On the other hand, the development of a satisfactory gas—liquid chromatographic (GLC) procedure for the analysis of taurine has been hindered by the difficulty of preparing a suitably volatile derivative of this compound. Although several investigators have reported that taurine can be chromatographed as its trimethylsilyl derivative [25, 26], to our knowledge no report has so far appeared on the actual GLC analysis of taurine contained in biological samples.

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This paper reports the development of a GLC method for the determination of taurine, based on the preparation of the N-isobutyloxycarbonyl (N-isoBOC) di-*n*-butylamide derivative.

## EXPERIMENTAL

### *Reagents*

Taurine and 3-amino-1-propanesulphonic acid (APS) as an internal standard were purchased from Nakarai Chemicals (Kyoto, Japan) and Sigma (St. Louis, MO, U.S.A.), respectively, and each was dissolved in water to make a stock solution at a concentration of 1 m*M*. Isobutyl chloroformate (isoBCF) stabilized with calcium carbonate was purchased from Eastman Kodak (Rochester, NY, U.S.A.). Thionyl chloride and di-*n*-butylamine (DBA) were purchased from Nakarai Chemicals and used after distillation. Tetrahexylammonium hydroxide (THA-OH) was prepared as follows: 10 g (0.02 mol) of tetrahexylammonium iodide (Eastman Kodak) were dissolved in 60 ml of 80% methanol and to this solution was added 0.07 mol of freshly precipitated silver oxide. The mixture was shaken for 1 h at room temperature. After centrifugation, the supernatant was evaporated at 50°C, and the residue was reconstituted in methanol to prepare a 10% (w/v) solution. Other alkylammonium hydroxide solutions were prepared in the same fashion as described above except for tetraethylammonium hydroxide and tetrabutylammonium hydroxide, which were obtained as 10% methanolic solutions from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals were of analytical grade.

### *Instruments*

A Shimadzu 4CM gas chromatograph equipped with a hydrogen flame ionization detector, an on-column injection port and a linear temperature programmer was used. The column packing, 1.5% OV-17 on 100—120 mesh Uniport HP (Gasukuro Kogyo, Tokyo, Japan), was prepared using toluene as a coating solvent according to the solution coating technique [27], and was poured into a silanized glass column (1.5 m × 3 mm I.D.). The packed column was conditioned at 290°C for 24 h with a nitrogen flow-rate of 30 ml/min. The operating conditions were as follows: oven temperature, programmed at 5°C/min from 200°C to 280°C; injection and detector temperatures, 285°C; nitrogen flow-rate, 45 ml/min. A Shimadzu-LKB 9000 gas chromatograph—mass spectrometer with the same type of column as used for GLC analysis was employed under the following conditions: trap current, 60 μA; ionizing voltage, 70 eV; accelerating voltage, 3.5 kV; ion source temperature, 290°C; separation temperature, 285°C; helium flow-rate, 30 ml/min.

### *Derivatization*

The chemical reactions involved in the derivatization procedure proceed as shown in Fig. 1. Pyrex glass screw-top culture tubes (10 cm × 1.0 cm I.D.) with PTFE-lined caps were used as reaction vials. An aliquot of the taurine solution (corresponding to 5—500 nmol) or 0.1 ml of 24-h human urine was pipetted into a 10-ml reaction vial. After addition of 0.2 ml of the internal standard solution, 0.1 ml of 0.5 *M* sodium hydroxide was added and then the total

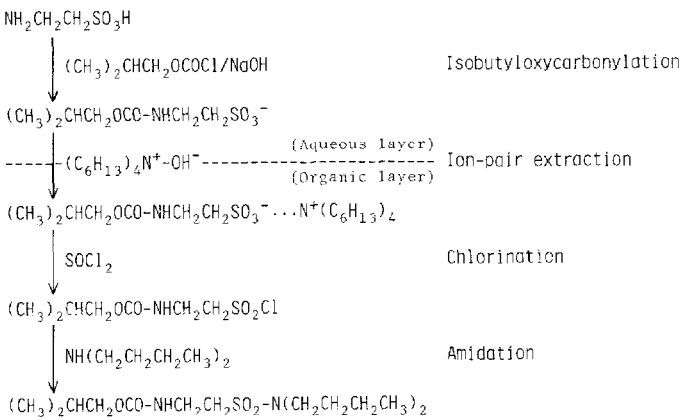


**Fig. 1. Derivatization process of taurine.**

reaction volume was made up to 1 ml with distilled water if necessary. Immediately after addition of 0.1 ml of isoBCF, the mixture was shaken with a shaker set at 300 rpm (up and down) for 5 min at room temperature. The reaction mixture was washed twice with 3 ml of diethyl ether after adjustment to pH 1–2 with 0.5 *M* hydrochloric acid. Subsequently, 0.1 ml of 10% THA-OH was added to the aqueous layer, and the ion-pair compounds formed were extracted into 2 ml of methylene chloride by shaking for 3 min. After centrifugation for 1 min, the organic layer was transferred to another reaction vial and the solvent was evaporated at 60° C under a stream of nitrogen. To the residue was added 0.2 ml of thionyl chloride, and the vial was tightly capped and heated at 80° C for 15 min. The excess thionyl chloride was removed at 80° C under a stream of dry nitrogen. To the residue was added 0.2 ml of 2 *M* DBA in acetonitrile, and the mixture was allowed to stand for 5 min at room temperature after tightly capping. The reaction mixture was acidified with 1 ml of 20% orthophosphoric acid and then extracted three times with 3 ml of *n*-pentane. After the solvent was evaporated to dryness at 60° C, the residue was dissolved in 0.1 ml of ethyl acetate and 2—4 μl of this solution were injected into the gas chromatograph. The peak height ratios relative to the internal standard were calculated, and they were used to construct the calibration curve and for the quantitation of taurine in urine.

*Preparation of reference compound*

A reference sample of the N-isoBOC di-*n*-butylamide derivative of taurine was prepared from 400 mg of taurine in essentially the same manner as the analytical derivatization procedure. The data for elemental analysis are as follows. Calc. for $C_{15}H_{32}N_2O_4S$: C, 53.54; H, 9.59; N, 8.33. Found: C, 53.21, H, 9.74; N, 8.11.

## RESULTS AND DISCUSSION

*Derivatization*

On the basis of the experience gained in our previous studies [28, 29], the isoBOC group was selected as the blocking substituent for the amino function

TABLE I

INFLUENCE OF THE NATURE OF THE COUNTER-ION ON ION-PAIR EXTRACTION OF N-isoBOC DERIVATIVE OF TAURINE

Reactants: counter-ion 1%, taurine 0.2 μmol, internal standard (9-bromophenanthrene) 0.2 μmol. GLC conditions are given in Experimental.

| Counter-ion | Number of C atoms | PHR* |
|---|---|---|
| Tetraethylammonium hydroxide | 8 | 0 |
| Tetrabutylammonium hydroxide | 16 | 0.49 |
| Trimethylstearylammonium hydroxide | 21 | 0.58 |
| Tetrahexylammonium hydroxide | 24 | 1.07 |
| Trioctylmethylammonium hydroxide | 25 | 0.91 |

*Peak height ratios are given relative to internal standard.

of taurine. The isobutyloxycarbonylation proceeded rapidly and quantitatively under the conditions described in Experimental. However, the presence of free sulphonic acid precluded the resulting N-isoBOC taurine in the aqueous medium being directly extracted into an organic solvent. In order to solve this problem, ion-pair extraction [30] was used. Of several alkylammonium ions tested, THA proved to be the most satisfactory counter-ion for the purpose (Table I), and with it rapid and quantitative ion-pair extraction of the N-isoBOC taurine into methylene chloride was achieved.

Experiments were conducted to find suitable reaction conditions for the chlorination of small amounts of N-isoBOC taurine; reaction at 80° C for 15 min with 0.2 ml of thionyl chloride proved to be adequate for maximal formation, as shown in Fig. 2. Excess reagent was removed under a stream of dry nitrogen to eliminate possible interference in the subsequent reaction.

A number of amines were evaluated to produce a sulphonamide derivative which is satisfactory in respect to reaction yield and GLC behaviour from the

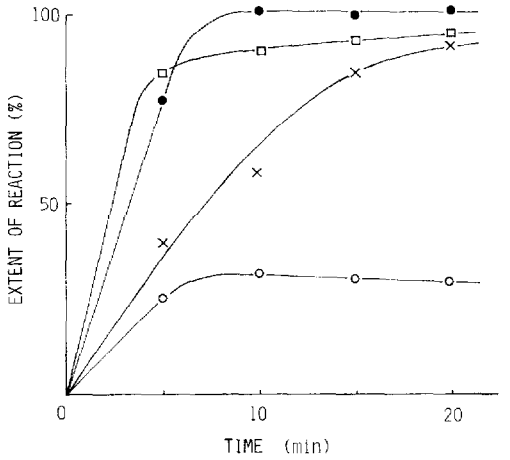


Fig. 2. Time dependence of chlorination of N-isoBOC taurine derivative at various temperatures: 40° C (○), 60° C (×), 80° C (●), 100° C (□).

N-isoBOC taurine sulphonyl chloride. The best result was obtained by DBA dissolved in anhydrous acetonitrile; the reaction proceeded rapidly without heating.

For extraction of the product, the N-isoBOC di-*n*-butylamide derivative, *n*-pentane was found to be a preferable solvent to others such as diethyl ether, ethyl acetate and benzene, the use of which resulted in the appearance of a large front peak on chromatograms, presumably derived from co-extracted THA.

The mean derivatization yield throughout the procedure established above was determined to be 97.3% ($n$ = 5) by comparison with the synthetic reference derivative.

*Structure and stability of derivative*

Structure of the derivative was confirmed both by gas chromatography—mass spectrometry (GC—MS) and by elemental analysis. Although a molecular ion peak with the postulated *m/e* 336 was not observed, the highest fragment ion peak with *m/e* 263 [$M^+ - (CH_3)_2CHCH_2O^+$] as well as other prominent fragment ion peaks [$M^+ - (CH_3)_2CHCH_2OCONH$, $M^+ - N(C_4H_9)_2$, $M^+ - (CH_3)_2CHCH_2$ and $N(C_4H_9)_2$] were useful for structure elucidation. The values of elemental analysis agreed with the theoretical values calculated for the structure expected. These results supported the structure for the derivative shown in Fig. 1. The derivative was found to be very stable under normal laboratory conditions; no decomposition was observed even after standing in ethyl acetate for three weeks at room temperature.

*Quantitative aspects*

The derivative of taurine gave an excellent peak on an OV-17 column (Fig. 3). The calibration curve for taurine was conducted using APS, which showed a similar behaviour to taurine during the chemical reactions and was well separated from taurine on a chromatogram as the internal standard. Its linearity was observed in the range 5—500 nmol of taurine, and the relative standard deviation at each point ranged from 1.5 to 5.2% ($n$ = 5), indicating that the reproducibility of analysis throughout the procedure is satisfactory.

*Application to the determination of urinary taurine*

In order to demonstrate the applicability of the method to biological materials, the content of taurine in human urine was analysed. A preliminary study indicated that the urinary components containing the carboxylic acid function such as amino acids and phenolic acids also provided respective peaks when they were converted into the corresponding isoBOC di-*n*-butylamide derivatives. However, these compounds could be clearly excluded as their isoBOC derivatives at the diethyl ether washing step of the analytical derivatization. Fig. 4 demonstrates the effectiveness of diethyl ether washing. Hippuric acid was also excluded by this washing. GC—MS analysis of the peaks of taurine and the internal standard from urine samples confirmed that each peak was almost uniform.

The recovery rates of taurine added to 0.1 ml of urine in the range 50—500 nmol were 98.0—105.8%, and their relative standard deviations were 1.2—

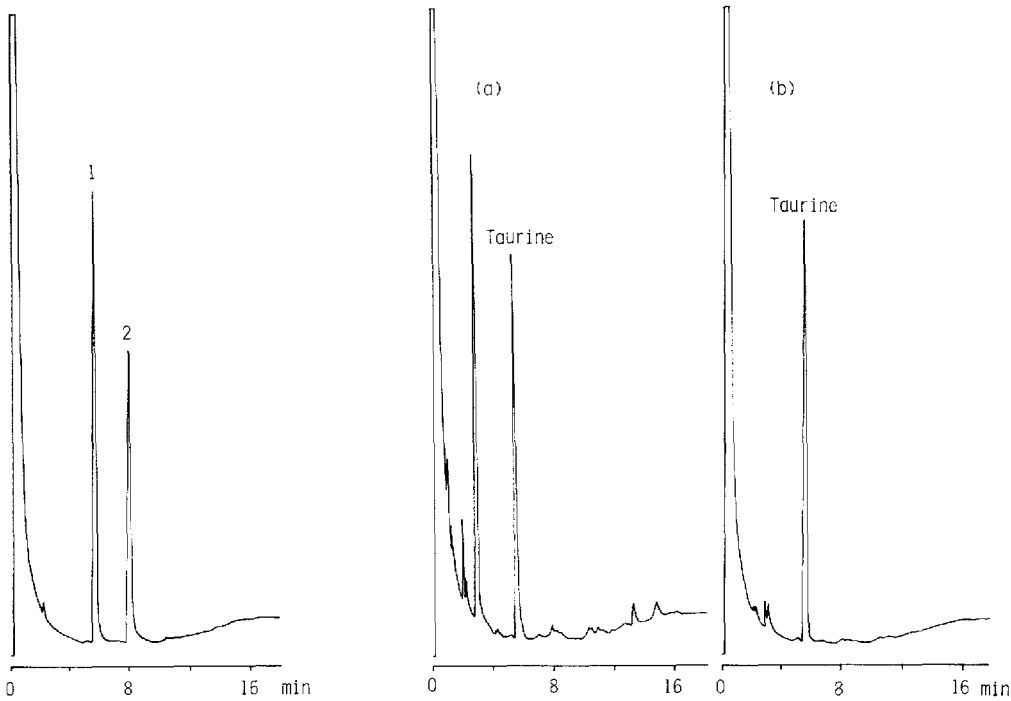


Fig. 3. Gas chromatogram obtained from a standard solution containing 250 nmol each of taurine and APS (internal standard). GLC conditions are given in Experimental. Peaks: 1 = taurine, 2 = APS.

Fig. 4. Gas chromatograms showing the effectiveness of diethyl ether washing on removal of interfering components in human urine: (a) without washing; (b) with washing. In each case, the injection aliquot is equivalent to 2 $\mu$l of the original urine and contains approximately 5 nmol of taurine.

TABLE II

RECOVERY OF TAURINE ADDED TO HUMAN URINE

| Taurine added ($\mu$mol/ml) | Amount found ($\mu$mol/ml), mean ± S.D.* ($n$ = 5) | R.S.D.** (%) | Recovery (%) |
|---|---|---|---|
| 0 | 2.03 ± 0.10 | 4.9 | |
| 0.5 | 2.53 ± 0.03 | 1.2 | 100.0 |
| 1.0 | 3.01 ± 0.09 | 3.0 | 98.0 |
| 2.0 | 4.01 ± 0.12 | 3.0 | 99.0 |
| 5.0 | 7.32 ± 0.15 | 2.0 | 105.8 |
| Mean ± S.D.* | | | 100.7 ± 3.5 |

*Standard deviation.
**Relative standard deviation.

TABLE III

URINARY EXCRETION OF TAURINE IN NORMAL SUBJECTS

| Sample number | Age (years) | Sex* | Taurine ($\mu$mol per 24 h)** |
|---|---|---|---|
| 1 | 9 | M | 499 |
| 2 | 21 | F | 2357 |
| 3 | 22 | F | 506 |
| 4 | 22 | F | 801 |
| 5 | 23 | M | 625 |
| 6 | 27 | M | 1260 |
| 7 | 36 | F | 1682 |
| 8 | 41 | M | 2147 |
| 9 | 43 | M | 1733 |
| 10 | 52 | M | 809 |

*M = male, F = female.
**Each value represents an average of three analyses.

4.9% ($n$ = 5), indicating that this method is very accurate and precise (Table II). The 24-h urinary concentrations obtained from ten healthy volunteers are shown in Table III.

In conclusion, these experiments have conclusively demonstrated that taurine can be accurately and precisely determined by GLC as its N-isoBOC di-$n$-butylamide derivative. Complex biological material such as urine can be analysed by this method without prior clean-up of the sample. We believe that this method provides a useful tool in biochemical and biomedical research requiring taurine assay.

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