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# Capillary gas chromatographic determination of proteins and biological amino acids as N(O)-*tert.*-butyldimethylsilyl derivatives

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**Abstract**

Forty-seven biological amino acids containing all 22 protein amino acids were derivatized to N(O)-*tert.*-butyldimethylsilyl (tBDMSi) derivatives by a single-step reaction with N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide and successfully separated on an HP-1 capillary column. The relative standard deviations of the relative molar responses of most amino acids were <5%. Cystine seems to be partially converted into cysteine during derivatization. An increase in carrier gas flow-rate towards the end of the analysis by inlet pressure programming with electron pressure control avoided the peak broadening and adsorption of the derivatives with high boiling points on the column and especially increased sensitivity of cystine to 5 pmol. Glutamine was converted almost completely into pyroglutamic acid during prolonged storage of a standard solution prepared in 0.01 *M* HCl but not during derivatization. These results compared with those for the phenylthiocarbamyl derivatives analysed by HPLC and the analytical results reported in the literature on soybean hydrolysate showed good agreement except for cysteine. The results for the amino acid composition of bovine serum albumin also showed good agreement with results in the literature except for cysteine. In human urine, seventeen free amino acids were detected as tBDMSi derivatives.

## 1. Introduction

The gas chromatographic analysis of amino acids has many advantages compared with other methods in terms of the sensitivity, cost, versatility of the instrument and combination of the system with mass spectrometry [1]. Moreover, in the determination of biological amino acids, the fast, sensitive and precise analysis and identification of resolved amino acid peaks are very important in diagnosing the diseases caused by disorders of protein metabolism or to elucidate amino acid metabolites. Such analytical criteria are perfectly matched to gas chromatographic (GC) methodology because this method permits gas chromatography–mass spectrometry (GC–MS) coupling, which offers a much better means of investigation than other analytical methods [2]. For the GC analysis of amino acids, however, volatile and stable derivatives are required.

The major derivatization methods developed in the past involve N-acyl alkyl esters [3–18] and trimethylsilyl (TMS) derivatives with bis-(trimethylsilyl)trifluoroacetamide (BSTFA) [19].

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However, in the case of N-acyl alkyl esters, the derivatization was carried out with more than two-step reactions and not all of the protein amino acids were derivatized by these methods or those of the TMS derivatives.

In recent years, the search for silyl derivatives alternative to TMS was an active area of investigation for enhancing the thermal and hydrolytic stability, the inertness of the analyte relative to the chromatographic system, separations in chromatographic analyses and sensitivity and selectivity when used with a selective detector, e.g., element-specific detectors or GC–MS with selected-ion monitoring. For these purposes, derivatization to N(O)-*tert.*-butyldimethylsilyl (tBDMSi) derivatives of many organic compounds using several reagents, such as *tert.*-butyldimethylchlorosilane (TBDMCS), N-*tert.*-butyldimethylsilylimidazole (TBDMSIM) and N-methyl - N - (*tert.* - butyldimethylsilyl)trifluoroacetamide (MTBSTFA) has already been accomplished. The advantages of using MTBSTFA rather than TBDMSIM and TBDMCS are as follows: (i) enhanced reactivity, including its ability to silylate carboxyls, hydroxyls, thiols and primary and secondary amines; (ii) short reaction times, often at room temperature; and (iii) simplified work owing to the derivatization by-product being neutral and volatile, thus providing the possibility for direct injection of the reaction mixture for GC analysis [20].

The silylating agent MTBSTFA has been successfully adopted for derivatization into tBDMSi derivatives all of the 22 protein amino acids by a one-step reaction and the derivatives were completely resolved on a single capillary column. Some of the properties of tBDMSi amino acid derivatives were advantageous with respect to the points discussed above, i.e. inertness, separation, thermal and hydrolytic stability, sensitivity and selectivity and the possibility to apply direct injection [1,21]. Biological amino acids were also analysed as such derivatives [21–23]. There are also other reports that not all of the 22 protein amino acids were derivatized into tBDMSi derivatives, especially cystine [24] and cysteine [23]. Glutamine was converted into pyroglutamic acid during derivatization [1,25], but in another report glutamine was derivatized to tBDMSi derivatives without conversion [22]. Substantial amounts of glutamine, asparagine and tryptophan were destroyed during derivatization [23,24] and derivatization of threonine was very difficult [26]. Although MTBSTFA is known as a superior derivatization reagent for amino acids, there is a surprising lack of papers on the biological amino acids and protein amino acids as tBDMSi derivatives in the real samples.

In this paper, we examine several of the problems indicated above and report the determination as tBDMSi derivatives, obtained using MTBSTFA, of the standard protein and biological amino acids, soybean and bovine serum albumin (BSA) hydrolysates and free amino acids in human urine.

## 2. Experimental

### 2.1. Material

MTBSTFA, triethylamine, N,N-dimethylformamide (DMF), pyridine and octadecane were obtained from Aldrich (Milwaukee, WI, USA). Standard amino acids and BSA were obtained from Sigma (St. Louis, MO, USA). The other reagents were of analytical-reagent grade.

### 2.2. Gas chromatography

All separations were carried out with an HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with electronic pressure control (EPC) and a flame ionization detector. The capillary column was an HP-1 (100% methylsiloxane) fused-silica column (50 m × 0.2 mm I.D., 0.25 $\mu$m film thickness) (Hewlett-Packard). The oven temperature was programmed as indicated in the captions of Figs. 1 and 2. The inlet pressure and carrier gas flow-rate were controlled with EPC. The inlet valve was also controlled with EPC, i.e., left off for 0.3 min after injection and then left on for the remaining time.

### 2.3. Sample preparation

Soybean meal (0.2 g) was placed into a 20-ml test-tube with an open-hole screw-cap with a

septum and 15 ml of 6 *M* HCl were added. BSA (2 mg) was placed in a 1-ml vial with the same type of cap and 0.2 ml of 6 *M* HCl was added. After tightly capping, the septum was pierced with two stainless-steel injection needles. One needle was connected to a nitrogen supply and the other to a vacuum pump. The test-tube and vial were evacuated with a vacuum pump for 2 min and then flushed with nitrogen. This step was repeated twice. The contents were hydrolysed at 145°C for 4 h. The BSA hydrolysate was dried with nitrogen at 50°C and dissolved in 5 ml 0.01 *M* HCl. This solution was used for derivatization. Soybean meal hydrolysate was filtered and dried in a rotary evaporator at 50°C. The residue was dissolved in 0.01 *M* HCl and the volume was adjusted to 50 ml. A 5-ml aliquot was passed through a 100 × 13 mm I.D. cation-exchange column (Dowex 5 × 8) and the retained amino acids were eluted with 3 *M* ammonia solution. The eluted solution was dried in a rotary evaporator at 50°C, dissolved in 0.01 *M* HCl, diluted to 50 ml and used for derivatization. For human urine, 20 ml of 15% sulfosalicylic acid were added to the same volume of urine and then allowed to stand for 1 h at 4°C and centrifuged at 5000 *g* for 15 min. A 5-ml volume of supernatant was purified using a cation-exchange column.

### 2.4. Derivatization

Pyridine and DMF containing octadecane (2.5 μmol/ml) with internal standard were used with solvents to dissolve the amino acids for derivatization. Standard biological and protein amino acids (2.5 μmol/ml of 0.01 *M* HCl) and sample solution were derivatized as follows. To a 1-ml conical vial with screw-cap with a PTFE-faced silicone-rubber septum, 10–15 μl of solution were added and dried with dry nitrogen at 40°C and then about 50 μl of acetonitrile were added and the mixture was dried again. In order, 30 μl of solvent, 20 μl of MTBSTFA and 2 μl of triethylamine were added. After capping tightly, the vial was heated at 75°C for 30 min. The

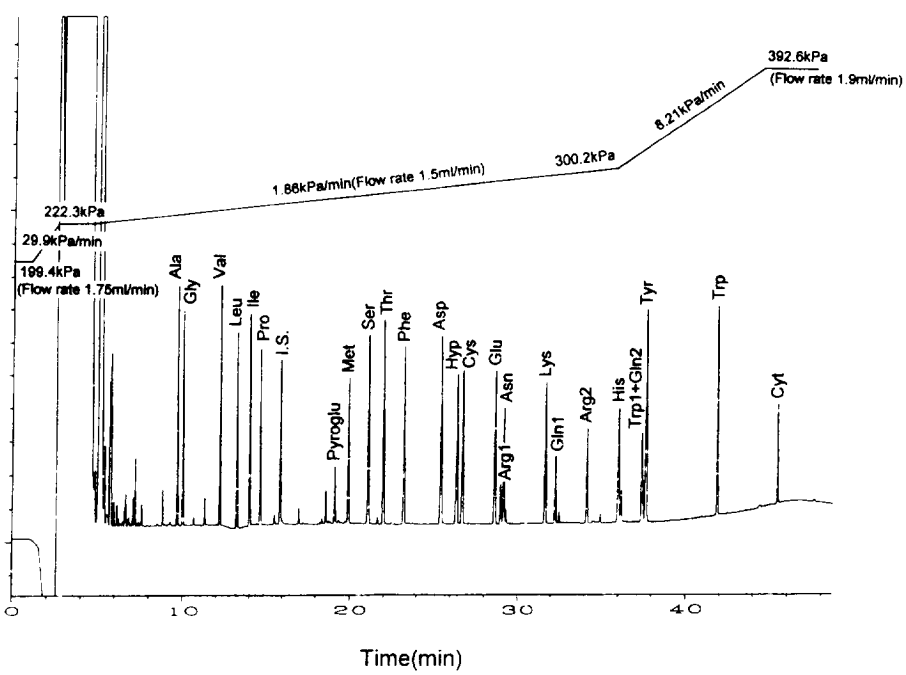


Fig. 1. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of standard protein amino acids. Temperature program: initial temperature, 40°C; 1-min hold, increased at 70°C/min to 145°C; 2-min hold; increased 3°C/min to 245°C and at 7°C/min to 300°C; 3-min hold. Injection and detector port temperature, 300°C; inlet value; 0.3-min purge off, remaining time purge on (splitless mode).

reaction mixture was cooled to room temperature and injected directly on to the GC column.

## Results and discussion

### 3.1. Chromatography

The chromatograms of the tBDMSi derivatives of the standard protein and biological amino acids derivatized in DMF as solvent are shown in Figs. 1 and 2. Forty-seven biological amino acids containing all 22 protein amino acids were derivatized to tBDMSi derivatives by a single-step reaction with MTBSTFA and successfully resolved on an HP-1 capillary column. The amino acids which showed two peaks were glutamine, arginine and tryptophan among the protein amino acids (Fig. 1) and citrulline among the biological amino acids (Fig. 2). Among the biological amino acids, the completely overlapped peaks were arginine 1 + ornithine + citrulline 1, DL-α-aminopimeric acid + citrulline 2 and glutamine 2 + tryptophan 1. With pyridine as solvent, tryptophan appeared as a single peak overlapped with glutamine 2 at the position of tryptophan 1 + glutamine 2 in DMF. Threonine and hydroxyproline showed very poor peak responses compared with the DMF solvent and proline showed two peaks. As a solvent, DMF was superior to pyridine. This phenomenon seems to be due to solubility properties, because

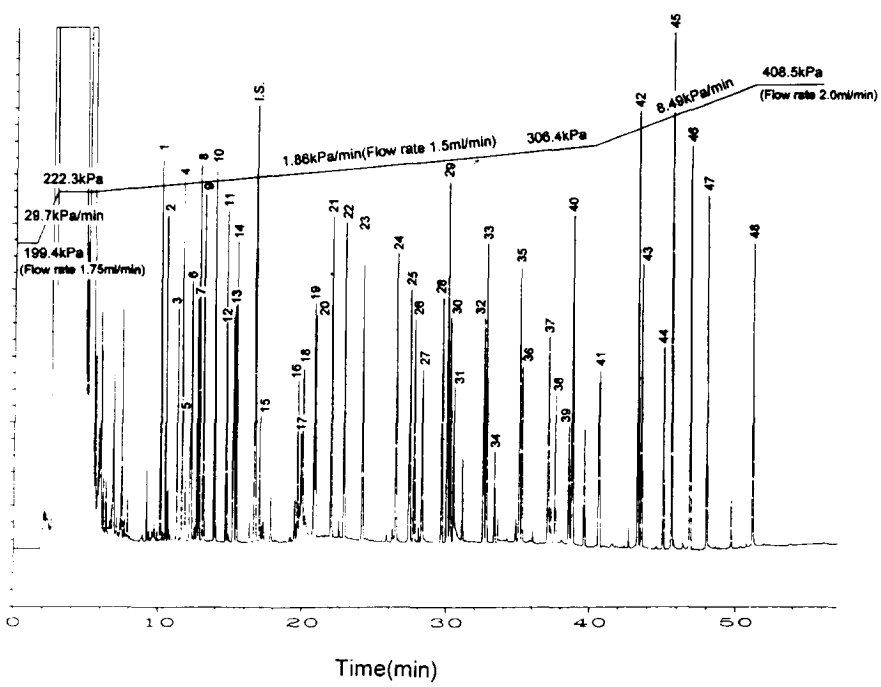


Fig. 2. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of standard biological amino acids. Temperature programme: initial temperature, 40°C; 1-min hold, increased at 70°C/min to 145°C; 3-min hold; increased at 3°C/min to 245°C and at 5°C/min to 305°C; 6-min hold. Peaks: 1 = Ala; 2 = Gly; 3 = orthophospho-L-serine; 4 = L-α-amino-*n*-butyric acid; 5 = urea; 6 = β-alanine; 7 = DL-β-aminoisobutyric acid; 8 = Val; 9 = L-norvaline; 10 = Leu; 11 = Ile; 12 = norleucine; 13 = γ-amino-*n*-butyric acid; 14 = Pro; 15 = sarcosine; 16 = orthophosphorylethanolamine; 17 = creatinine; 18 = pyroglutamic acid; 19 = Met; 20 = ε-amino-*n*-caproic acid; 21 = Ser; 22 = Thr; 23 = Phe; 24 = Asp; 25 = Hyp; 26 = Cys; 27 = 3-methyl-L-histidine; 28 = Glu; 29 = Arg 1 + ornithine + citrulline-1; 30 = Asn; 31 = 1-methyl-L-histidine; 32 = L-α-aminoadipic acid; 33 = Lys; 34 = Gln 1; 35 = DL-α-aminopimeric acid + citrulline 2; 36 = Arg 2; 37 = His; 38 = carnosine; 39 = Gln 2 + Trp 1; 40 = Tyr; 41 = δ-hydroxylysine; 42 = DL-ε-diaminopimelic acid; 43 = Trp 2; 44 = lanthionine; 45 = 3-(3,4-dihydroxyphenyl)-DL-alanine (DOPA); 46 = cystathionine; 47 = Cyt; 48 = homocystine.

the polarity of DMF is slightly greater than that of pyridine. With DMF as solvent, we could not find any artefact peaks that did not appear with pyridine except that tryptophan showed two peaks. Glutamine was converted into pyroglutamic acid almost completely during prolonged storage in 0.01 *M* HCl, but during derivatization a very small amount that did not affect the determination was converted into pyroglutamic acid (Fig. 3).

### 3.2. Relative molar responses (RMRs)

The relative standard deviations (R.S.D.s) of the RMR with respect to the internal standard were less than 5% for most derivatives (Table 1). Among the protein amino acids, only cystine

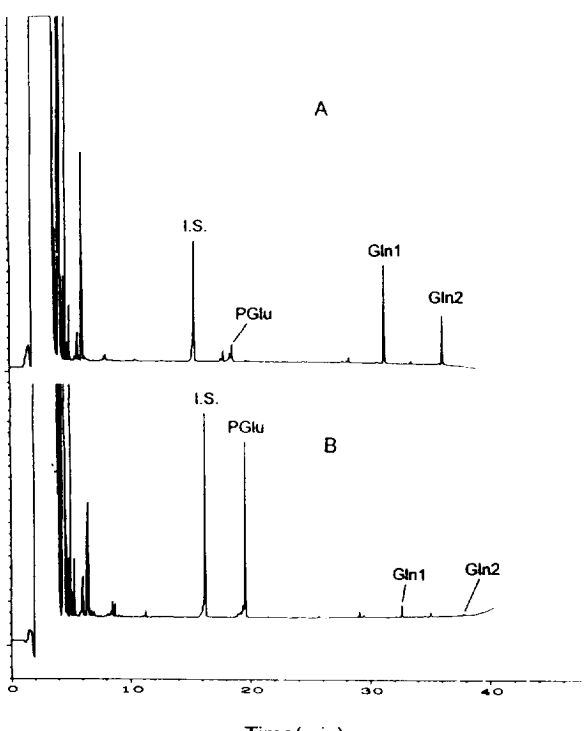


Fig. 3. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of glutamine. (A) Immediately derivatized glutamine after preparation of standard solution with 0.01 *M* HCl; (B) pyroglutamic acid (PGlu) derivative peak resulting from conversion of glutamine into pyroglutamic acid on prolonged storage of glutamine standard solution prepared with 0.01 *M* HCl.

Table 1
Relative molar responses (RMR) of N(O)-*tert.*-butyldimethylsilyl (tBDMSi) amino acid derivatives

| Amino acid | RMR[a] | S.D.[b] | R.S.D. (%)[b] |
|---|---|---|---|
| Alanine | 1.18 | 0.03 | 3.1 |
| Glycine | 1.06 | 0.03 | 2.2 |
| Orthophospho-L-serine | 0.47 | 0.02 | 4.3 |
| L-α-Amino-*n*-butyric acid | 1.18 | 0.05 | 4.2 |
| Urea | 0.57 | 0.01 | 1.8 |
| β-Alanine | 1.21 | 0.05 | 4.1 |
| DL-β-Aminoisobutyric acid | 0.94 | 0.01 | 1.1 |
| Valine | 1.32 | 0.01 | 1.1 |
| L-Norvaline | 1.28 | 0.02 | 1.6 |
| Leucine | 1.44 | 0.02 | 1.4 |
| Isoleucine | 1.34 | 0.02 | 1.5 |
| L-Norleucine | 1.49 | 0.09 | 6.0 |
| γ-Amino-*n*-butyric acid | 0.63 | 0.02 | 3.2 |
| Proline | 1.01 | 0.01 | 1.0 |
| Sarcosine | 0.58 | 0.01 | 1.7 |
| Orthophosphorylethanolamine | 0.51 | 0.01 | 2.0 |
| Creatinine | 0.92 | 0.01 | 1.1 |
| Pyroglutamic acid | 0.38 | 0.01 | 2.6 |
| Methionine | 0.80 | 0.02 | 2.5 |
| ε-Amino-*n*-caproic acid | 0.78 | 0.02 | 2.5 |
| Serine | 1.49 | 0.05 | 3.4 |
| Threonine | 1.51 | 0.06 | 4.0 |
| Phenylalanine | 1.46 | 0.04 | 2.7 |
| Aspartic acid | 1.40 | 0.05 | 3.6 |
| Hydroxyproline | 1.21 | 0.02 | 1.7 |
| Cysteine | 0.84 | 0.02 | 2.4 |
| 3-Methyl-L-histidine | 0.89 | 0.04 | 4.5 |
| Glutamic acid | 0.85 | 0.03 | 3.5 |
| Arginine 1 + ornitine + citrulline 1 | 1.59 | 0.07 | 4.4 |
| Asparagine | 0.71 | 0.03 | 4.2 |
| 1-Methyl-L-histidine | 0.32 | 0.02 | 6.3 |
| L-α-Aminoadipic acid | 1.05 | 0.05 | 4.8 |
| Lysine | 1.04 | 0.04 | 3.8 |
| Glutamine 1 | 0.23 | 0.01 | 4.3 |
| DL-α-Aminopimeric acid + citrulline 2 | 0.93 | 0.04 | 4.3 |
| Arginine 2 | 0.65 | 0.02 | 3.1 |
| Histidine | 0.85 | 0.04 | 4.7 |
| Carnosine | 0.33 | 0.01 | 3.0 |
| Tyrosine | 0.90 | 0.01 | 1.1 |
| δ-Hydroxylsine | 0.37 | 0.02 | 5.4 |
| DL-α,ε-Diaminopimelic acid | 1.20 | 0.04 | 3.3 |
| Tryptophan 2 | 0.92 | 0.03 | 3.3 |
| Lanthionine | 0.30 | 0.01 | 3.3 |
| 3-(3,4-Dihydroxyphenyl)-DL-alanine | 1.35 | 0.02 | 1.5 |
| Cystathionine | 1.02 | 0.02 | 2.0 |
| Cystine | 0.98 | 0.05 | 5.1 |
| Homocystine | 0.80 | 0.02 | 2.5 |

[a] Values are relative to octadecane = 1.
[b] $n = 4$.

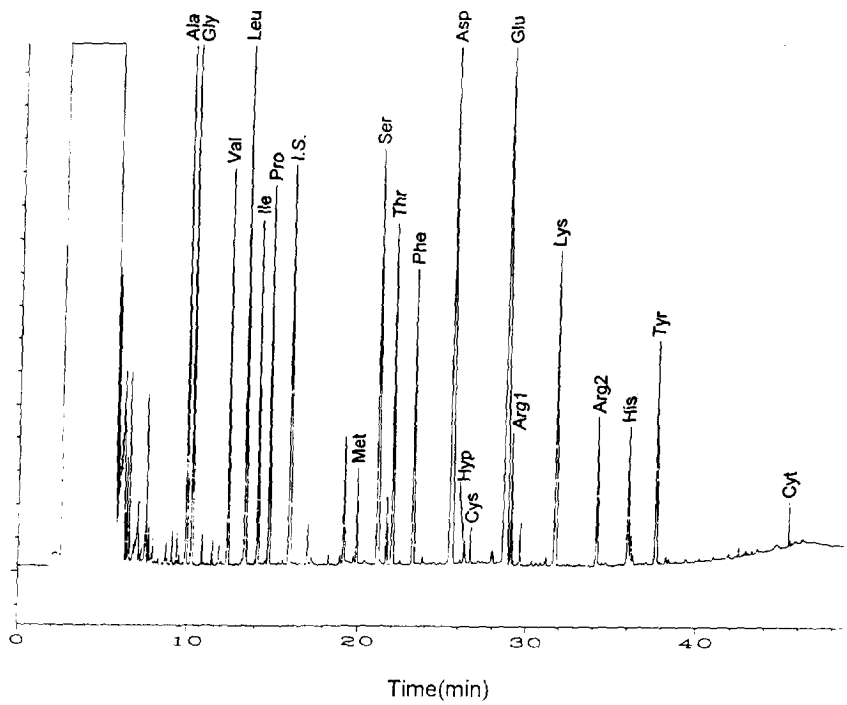


Fig. 4. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of amino acids in soybean hydrolysate. Gas chromatographic conditions as for standard protein amino acids.

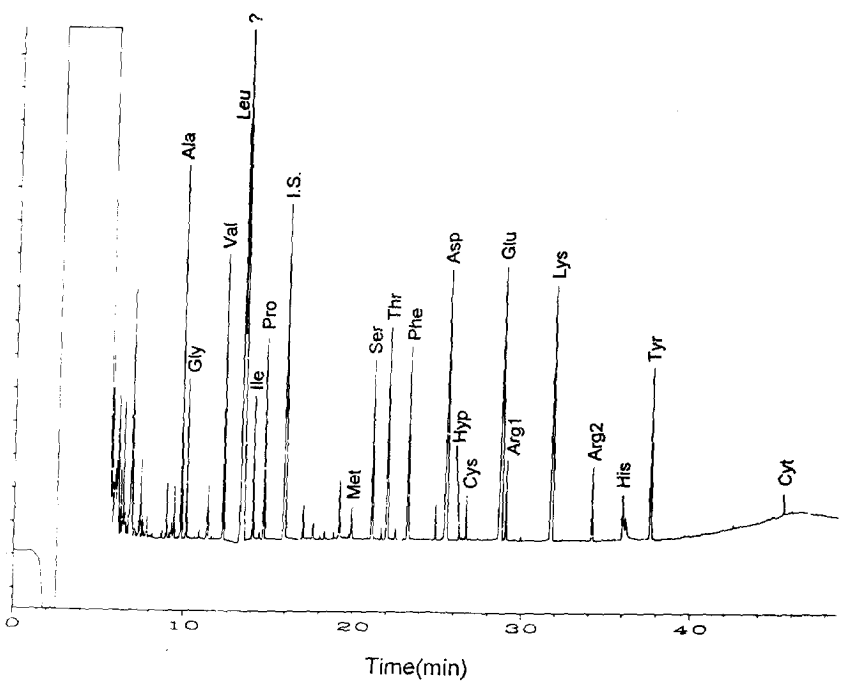


Fig. 5. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of amino acids in bovine serum albumin hydrolysate. Gas chromatographic conditions as for standard protein amino acids (Fig. 1).

exceeded R.S.D. 5%. Among the biological amino acids, L-norleucine, 1-methyl-L-histidine and δ-hydroxylysine exceeded R.S.D. 5% but not 10%. The derivatives of 1-methyl-L-histidine, glutamine, carnosine, δ-hydroxylysine and lanthionine showed low RMRs compared with other derivatives. In a previous study [1], the cystine peak was so small that it could not be detected below 400 pmol. However, in this study the response of cystine was not low compared with other amino acids and it could be detected at the level of 5 pmol. We think that this is caused by the flow-rate programming by EPC. An increase in carrier gas flow-rate towards the end of the analysis seems to avoid peak broadening and adsorption of the derivatives with high boiling points on the column.

### *3.3. Amino acid composition of soybean and bovine serum albumin*

Chromatograms of amino acids in soybean and BSA hydrolysates are shown in Figs. 4 and 5. Nineteen amino acids, i.e. excluding glutamine, asparagine and tryptophan which were completely destroyed by HCl hydrolysis, were detected in both samples. Especially hydroxyproline, which has not been reported in the literature, was detected in both samples and cystine was also detected in the BSA hydrolysate. Even though we did not identify these peaks by GC–MS, we think that they could be detected because of the high sensitivity of the GC method.

The results for the amino acid composition of both samples showed good agreement with li-

Table 2
Amino acid composition of soybean determined by the N(O)-*tert.*-butyldimethylsilyl (tBDMSi) derivatives method compared with the use of phenylthiocarbamyl (PTC) derivatives and the analytical results reported in the literature

| Amino acid | tBDMSi derivative | PTC derivative[a] (HPLC) | Concentration (g per 100 g dry matter) | |
|---|---|---|---|---|
| | | | Food composition table[b] | INFIC[c] |
| Ala | 1.90 ± 0.08[d] | 1.89 ± 0.35[d] | 1.88 | 1.51 |
| Gly | 1.70 ± 0.08 | 2.09 ± 0.13 | 1.75 | 1.64 |
| Val | 2.09 ± 0.12 | 2.12 ± 0.10 | 1.97 | 1.61 |
| Leu | 3.25 ± 0.33 | 3.10 ± 0.24 | 3.34 | 2.85 |
| Ile | 1.99 ± 0.28 | 1.83 ± 0.16 | 1.96 | 1.66 |
| Pro | 2.01 ± 0.10 | 2.38 ± 0.18 | 2.24 | 1.71 |
| Met | 0.52 ± 0.05 | 0.50 ± 0.25 | 0.56 | 0.51 |
| Ser | 1.93 ± 0.18 | 2.03 ± 0.43 | 2.09 | 1.98 |
| Thr | 1.82 ± 0.04 | 1.79 ± 0.05 | 1.67 | 1.77 |
| Phe | 2.35 ± 0.14 | 2.63 ± 0.18 | 2.30 | 1.82 |
| Asp | 4.46 ± 0.06 | 4.72 ± 0.63 | 4.92 | 4.37 |
| Hyp | 0.11 ± 0.03 | - | - | - |
| Cys | 0.14 ± 0.02 | 0.11 ± 0.01 | - | 1.66 |
| Glu | 7.29 ± 0.10 | 7.76 ± 0.84 | 8.00 | 7.30 |
| Arg | 2.91 ± 0.17 | 2.65 ± 0.36 | 2.89 | 3.21 |
| Lys | 2.64 ± 0.19 | 3.73 ± 0.25 | 2.64 | 2.56 |
| His | 1.13 ± 0.05 | 1.15 ± 0.04 | 1.13 | 1.04 |
| Tyr | 1.00 ± 0.08 | 1.26 ± 0.11 | 1.11 | 1.04 |
| Cyt | 0.56 ± 0.05 | - | 0.55 | 0.52 |

[a] Results determined with PTC derivatives on the same sample by reversed-phase HPLC on a Nova-Pak $C_{18}$ column.
[b] Ref. [27].
[c] Ref. [28].
[d] Mean ± S.D., $n = 4$.

terature results (Tables 2 and 3) or the results for the PTC derivatives method (Table 2) except for cysteine. The cysteine content in both samples was very low compared with the values reported in the literature (Tables 2 and 3). To elucidate this, we examined the destruction of standard cysteine and cystine by hydrolysis in 6 *M* HCl for 4 h at 145°C. About 25–65% of cysteine and 10–20% of the cystine were destroyed (Figs. 6C and 7C). During prolonged storage of the standard solution of cysteine prepared in 0.01 *M* HCl, a substantial amount of cysteine seems to be converted into cystine but not during the HCl hydrolysis (Fig. 6B and C). This phenomenon seems to be caused by the readily oxidizable property of cysteine [32]. Thus cysteine standard, and also glutamine, must be prepared just before analysis. Cystine was not destroyed during prolonged storage but it was partially and proportionally converted into cysteine during derivatization (Fig. 7). Cysteine did not show any conversion during derivatization (Fig. 6A). We therefore concluded that cystine and cysteine determinations have unavoidable error unless they are converted prior to quantification [33].

### 3.4. Biological amino acids in human urine

A typical chromatogram obtained for a normal human urine is illustrated in Fig. 8. We could detect thirteen common protein amino acids and four biological amino acids. A large amount of urea was also detected. 3-Methylhistidine and

Table 3
Amino acid composition of bovine serum albumin determined by the N(O)-*tert.*-butyldimethylsilyl (tBDMSi) derivatives method compared with the analytical results reported in the literature

| Amino acid | tBDMSi derivative[a] | Starch chromatography[c] | Automatic analyser[d] | Sequence[e] |
|---|---|---|---|---|
| Ala | 47.5 ± 1.12[b] | 47.4 | 45.2 | 46 |
| Gly | 16.0 ± 0.21 | 16.4 | 15.3 | 15 |
| Val | 36.3 ± 2.12 | 34.2 | 35.4 | 36 |
| Leu | 62.8 ± 0.75 | 63.3 | 59.2 | 61 |
| Ile | 13.7 ± 1.01 | 13.5 | 14.1 | 14 |
| Pro | 29.9 ± 1.83 | 27.9 | 28.5 | 28 |
| Met | 4.2 ± 0.58 | 3.6 | 3.8 | 4 |
| Ser | 30.1 ± 1.46 | 27.3 | 26.9 | 28 |
| Thr | 35.8 ± 5.37 | 33.1 | 33.0 | 34 |
| Phe | 31.0 ± 2.11 | 27.0 | 26.1 | 26 |
| Asp | 51.4 ± 1.27 | 55.4 | 55.0 | 53 |
| Hyp | 1.9 ± 0.61 | – | – | – |
| Cys | 16.3 ± 5.07 | 36.7 | 36.0 | 35 |
| Glu | 77.8 ± 1.21 | 75.8 | 82.0 | 78 |
| Lys | 61.7 ± 2.97 | 59.3 | 59.1 | 59 |
| Arg | 22.7 ± 0.62 | 22.9 | 23.6 | 23 |
| His | 18.1 ± 1.97 | 17.4 | 17.0 | 17 |
| Tyr | 19.8 ± 1.96 | 18.9 | 19.9 | 19 |
| Trp | – | 1.9 | 2.1 | 2 |
| Cyt | 5.2 ± 0.50 | – | – | – |
| Total | 582.2 | 582.0 | 582.2 | 578 |

[a] $n = 3$.
[b] The values are recalculated on the basis of a total of 582 residues.
[c] Ref. [29].
[d] Ref. [30].
[e] Ref. [31].

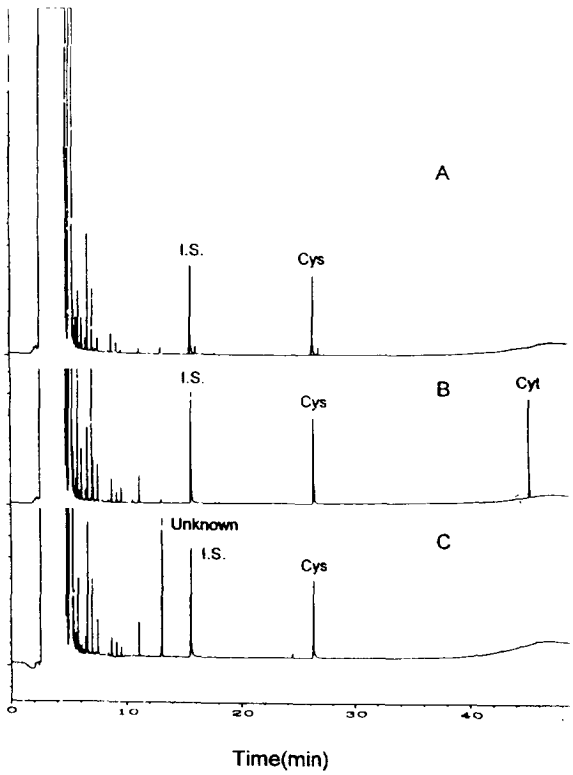


Fig. 6. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of cysteine. (A) Immediately derivatized cysteine after preparation of standard solution with 0.01 *M* HCl; (B) cysteine peaks derivatized with the standard solution stored for a long time; (C) cysteine peaks derivatized with cysteine hydrolysed in 6 *M* HCl for 4 h at 145°C.

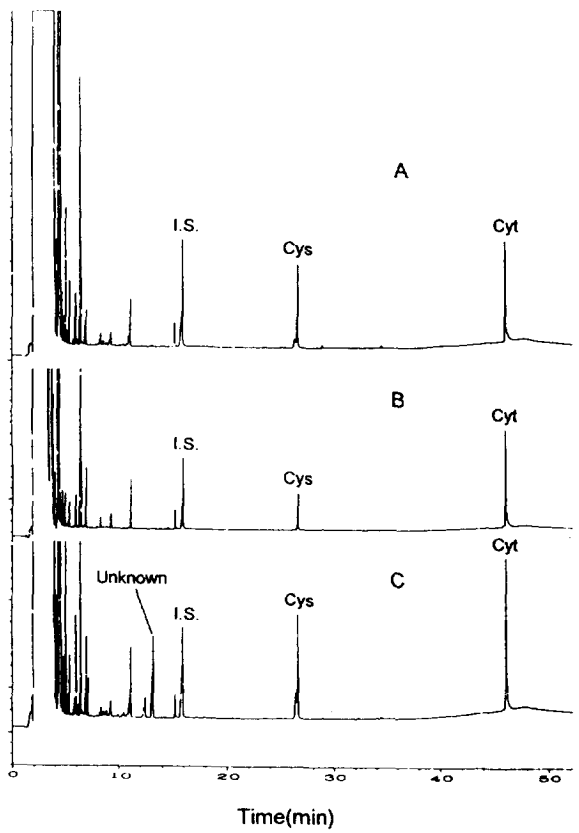


Fig. 7. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of cystine. (A) Immediately derivatized cystine after preparation of standard solution with 0.01 *M* HCl; (B) cystine peaks derivatized with the standard solution stored for a long time; (C) cystine peaks derivatized with cystine hydrolysed in 6 *M* HCl for 4 h at 145°C.

creatinine, related to muscle degradation and exercise, were detected. The retention time of the peak confirmed as ornithine, an intermediate of the urea cycle, is the same as that of arginine 1 and citrulline 1. However, we could conclude that this peak was ornithine because an arginine 2 or citrulline 2 peak, that have larger peak responses than arginine 1 or citrulline 1, were not detected. We also examined the possibility of overlap of other amino acid peaks with the urea peak because of the large amount of urea in urine. The urea in the urine was hydrolysed with urease for 4 h at 25°C and then the subsequent procedures were the same as those for the sample. The chromatogram was the same as that of the sample not treated with urease, except that the urea peaks (two peaks) disappeared. It therefore appeared that there were no overlapped peaks with urea.

## 4. Conclusion

N(O)-*tert.*-Butyldimethylsilyl derivatization with MTBSTFA is a useful method for the determination of protein and biological amino acids. The results for soybean and BSA determined with this method showed good agreement with those of the HPLC method with PTC derivatives and other reports, except for cysteine. Cysteine and cystine could not be

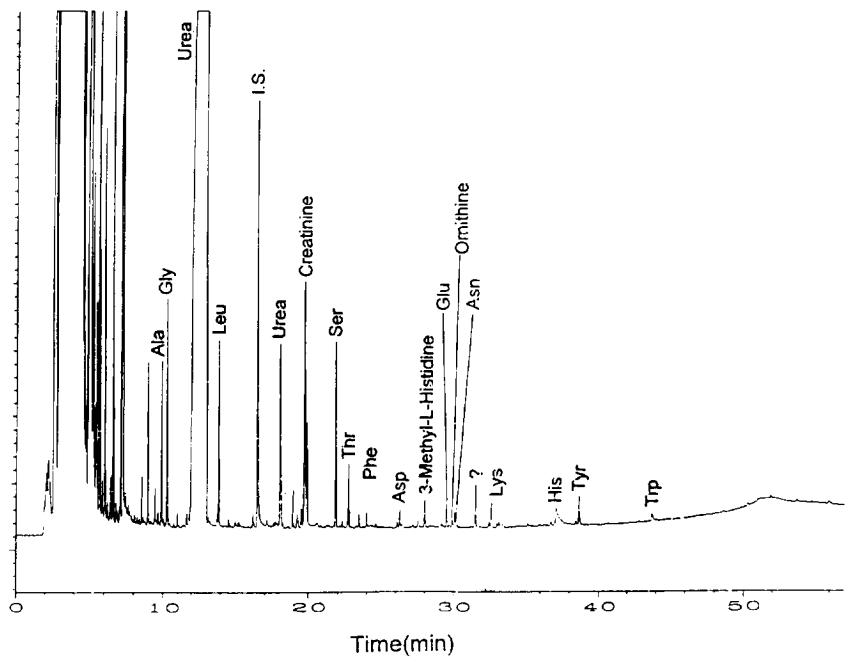


Fig. 8. Gas chromatogram of N(O)-*tert.*-butyldimethylsilyl derivatives of free amino acids in human urine. Gas chromatographic conditions as for standard biological amino acids (Fig. 2).

determined accurately because of the partial conversion of cystine into cysteine during derivatization. Glutamine was not converted into pyroglutamic acid by derivatization but it was completely converted into pyroglutamic acid during prolonged storage in 0.01 *M* HCl. In human urine, biological amino acids related to muscle degradation and exercise, 3-methylhistidine and creatinine, were clearly detected with other free amino acids.

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