Electron ionization and atmospheric pressure photochemical ionization in gas chromatography-mass spectrometry analysis of amino acids

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The mass spectra of *tert*-butyldimethylsilyl (TBDMS) derivatives of 17 amino acids were obtained using electron ionization (EI) and atmospheric pressure photochemical ionization (APPhCI) mass spectrometry. The APPhCI mass spectra for all of the derivatives except arginine were shown to consist of only molecular $[M]^{+\cdot}$ and quasimolecular $[MH]^+$ ions whereas, in the case of EI, the compounds in question underwent a drastic fragmentation. The application of APPhCI to gas chromatography-mass spectrometry enables a reliable identification of the TBDMS derivatives of amino acids in a mixture, even if its components are only partially resolved, due to the unique molecular masses for each compound. Comparison of the respective positive-ion chemical ionization (PICI) mass spectra available in the literature with APPhCI spectra has shown that, in the case of PICI, unlike APPhCI, noticeable fragmentation occurs.

Keywords: electron ionization, atmospheric pressure photochemical ionization, amino acids, silylation, TBDMS derivatives, MTBSTFA

Introduction

The most common and widely used procedure in gas chromatographic analysis of amino acids is silylation with different reagents. Formerly, *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was utilized in a number of the pioneering works.[1-3] However, the reaction required an elevated temperature (above the solvent boiling point) and special care to avoid any residual amount of water, since trimethylsilyl (TMS) esters of amino acids are known to be unstable and very susceptible to hydrolysis. In addition, there were no universal conditions for the simultaneous silylation of all amino acids: 15 min was enough for most of them, but some amino acids required 4 h of heating for the reaction to proceed quantitatively.

Recently, *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) has been introduced into the practice of gas chromatographic analysis of amino acids. It should be noted that various authors[4-9] have proposed different derivatization conditions (a solvent to be used, the temperature and time of the reaction), but the *tert*-butyldimethylsilyl (TBDMS) derivatives are formed under milder conditions and have been demonstrated to be much more stable to moisture. At the same time, according to Reference 5, molecular ions are practically absent in the electron ionization (EI) mass spectra of the TBDMS derivatives of amino acids; a fact that complicated their identification and quantitation in complex mixtures.

Unlike EI, atmospheric pressure photoionization (APPhI) and atmospheric pressure photochemical ionization (APPhCI) mass spectrometry, developed by us,[10,11] enable the registering of mass spectra for individual compounds, which consist of only molecular $[M]^{+\cdot}$ and/or quasimolecular $[MH]^+$ ion peaks. This was demonstrated for *n*-alkanes, alcohols, esters, ethers and amines which gave little or no molecular ion peak in their EI mass spectra. APPhI MS and APPhCI MS were coupled to capillary GC,[10-17] and the respective mass spectra were registered for a wide range of compounds (aromatic hydrocarbons, polyaromatic hydrocarbons [PAHs], normal and branched alcohols, phthalates,

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phenols, amines, phosphates, nitroaromatics, polychlorobiphenyls [PCBs] etc). The total number of investigated compounds was more than 100 and, in all cases, APPhI and APPhCI mass spectra consisted of only molecular and/or quasimolecular ion peaks, depending on the nature of the compound and reagent vapor used in APPhCI MS. Direct photoionization in APPhI GC-MS resulted mainly in formation of the molecular ions whereas, in the case of APPhCI GC-MS, both molecular and quasimolecular ions were produced due to ion–molecule reactions involving charge and proton transfer.

Detection limits (S:N = 3) in APPhI GC-MS varied from 10^{-10} g to 10^{-12} g in the selected-ion monitoring (SIM) mode, depending on the ionization potential of a compound and the UV-lamp energy. For *m*-xylene it was equal to 1×10^{-12} g. In the case of APPhCI GC-MS, the detection limit for *m*-xylene using toluene vapor as reagent gas was 4×10^{-14} g in the SIM mode. For benzophenone, the detection limit was about 10^{-14} g.[10] At the same time, detection limits in APPhCI GC-MS for surrogates of organophosphorus chemical warfare agents such as tripropylphosphate, diisoamylmethylphosphonate, dipinacolylmethylphosphonate and tributylphosphate, when working in the SIM mode, were 8×10^{-14}–3.3×10^{-13} g, depending on the compound. Detection limits for APPhI GC-MS in the SIM mode were about 100 times greater.[18] It was also shown that detection limits in APPhCI GC-MS for other investigated compounds were 20–110 times lower than in APPhI GC-MS (depending on the compound, using the same reagent gas). Vapors of such ultrapure compounds as benzene, toluene and acetone of "GC Standard" quality have been tested as reagent gases.

In the case of mixture analysis without separation,[11–17,19–22] APPhCI MS was mainly applied, using a solvent vapor as reagent gas. APPhCI mass spectra consisted of a number of peaks that coincided exactly with the number of components in the mixture, and every peak corresponded to the molecular or quasimolecular ion. Detection limits in this case were close or equal to those obtained in APPhCI GC-MS. APPhI MS ionization conditions, in the case of mixture analysis without separation, corresponded to conditions used in APPhCI MS and APPhI MS coupled to high-performance liquid chromatography (HPLC) or to the flow-injection technique, when eluant and eluates were fully evaporated before they reached the photoionization area. It goes without saying that this method includes opportunities for the so-called "dopant" method,[23] in which dopant ionizable compound is added to a vapor. It is also clear that direct mixture mass spectrometric analysis[23–26] using photoionization at atmospheric pressure or subatmospheric pressure is, in reality, APPhCI MS or subAPPhCI MS.

We have coupled atmospheric pressure photochemical ionization with microliquid chromatography.[27] However, we considered that, for compounds which could be analyzed by both APPhCI HPLC-MS and APPhI/APPhCI GC-MS, the latter was much more preferable due to the higher efficiency of HRGC and to the higher sensitivity of APPhCI GC-MS compared with APPhCI HPLC-MS. In addition, it is necessary to note the higher reliability of unknown component number recognition by APPhCI GC-MS, compared with APPhCI HPLC-MS, owing to the exclusion of side ion–molecule reactions with eluant impurities. It is well-known that, due to this reaction, some mixture components cannot be registered.

Direct analysis without separation was used in our new approach to quality control of chemical and pharmaceutical products.[13–17,21,22] It was based on direct analysis of liquid products or solutions of solids by APPhCI MS, APPhI GC-MS and APPhCI GC-MS using molecular weights of mixture components registered, without separation, by APPhCI MS. In this way, we were able to register co-eluting compounds using the respective mass chromatograms. The number of impurities found in investigated products using this new approach was 10–25 times more in comparison with HPLC-MS or GC-MS EI methods.

The combined use of APPhCI MS for mixture analysis without separation along with APPhI GC-MS and APPhCI GC-MS has greatly increased the reliability of component number recognition in complex mixtures and allowed the determination of co-eluting compounds with their molecular weights. The latter is very important for proper identification of mixture components based on EI, as the EI mass spectra for many classes of compounds do not contain a molecular ion peak or its intensity is negligible.

Reliable identification and quantitation of amino acids in complex microbial mixtures is currently of great importance. Therefore, it was interesting to investigate the APPhCI mass spectra of the TBDMS derivatives of 17 amino acids. The aim of the present work was to study and compare the EI and APPhCI mass spectra of these TBDMS derivatives and to estimate their detection limits.

Experimental

GC-MS

The APPhCI investigation was carried out using a GC-MS (Finnigan, model 4021) equipped with an ion source for atmospheric pressure photoionization and photochemical ionization of our own design (Instrument 1). These methods of ionization are described in References 10 and 11. The capillary column was connected directly to the ion source of approx. 150 µL volume; a make-up gas (helium) flow was also used. The pressure in the ion source was equal to atmospheric pressure.

A gas chromatograph (Trace GC) coupled to a mass-spectrometer (ThermoQuest Automass Multi) (Instrument 2) was used to register the EI mass spectra at 70 eV. A UV lamp with a photon energy of 10.6 eV was utilized for photoionization in APPhI/APPhCI MS operation.

The gas chromatographic conditions were as follows: injector temperature, 240°C; ion source temperature, 220°C;

Table 1. EI mass spectra of TBDMS derivatives of the amino acids.

Derivative	Molecular weight	*m/z* (I, %)
Ala (2TBDMS)	317	158 (100), 232 (46), 260 (32), 302 (2)
Gly (2TBDMS)	303	103 (13), 115 (7), 133 (14), 189 (21), 218 (100), 246 (74), 288 (3)
Val (2TBDMS)	345	133 (7), 186 (100), 216 (2), 260 (25), 288 (20), 302 (9), 330 (1)
Leu (2TBDMS)	359	123 (6), 200 (100), 274 (29), 302 (24)
Ile (2TBDMS)	359	133 (7), 200 (100), 274 (26), 302 (31)
Pro (2TBDMS)	343	184 (100), 258 (14), 286 (9)
Asn (3TBDMS)	474	116 (32), 158 (40), 174 (30), 244 (32), 302 (35), 417 (100)
Met (2TBDMS)	377	103 (8), 114 (7), 133 (14), 160 (7), 170 (27), 188 (8), 218 (100), 244 (15), 292 (62), 320 (6)
Ser (3TBDMS)	447	100 (18), 115 (27), 133 (32), 142 (18), 174 (12), 189 (8), 230 (29), 258 (8), 288 (96), 302 (49), 362 (100), 390 (92), 432 (6)
Thr (3TBDMS)	461	103 (36), 115 (38), 133 (15), 159 (58), 246 (7), 303 (100), 376 (27), 404 (35), 417 (10), 446 (3)
Phe (2TBDMS)	393	91 (13), 103 (11), 115 (14), 133 (21), 160 (17), 234 (96), 302 (100), 308 (51), 336 (51)
Lys (3TBDMS)	488	170 (48), 198 (100), 272 (45), 300 (99), 329 (31), 431 (27), 488 (14)
Arg (4TBDMS)	630	184 (100), 258 (30), 286 (40), 442 (60)
His (3TBDMS)	497	196 (100), 280 (9), 302 (12), 338 (43), 440 (75), 482 (4)
Tyr (3TBDMS)	523	133 (9), 160 (7), 221 (12), 245 (4), 302 (100), 364 (17), 438 (12), 466 (23), 508 (3)
Trp (2TBDMS)	432	130 (70), 218 (6), 245 (9), 273 (11), 302 (100), 347 (9), 375 (45)
Cys (4TBDMS)	696	100 (52), 132 (45), 188 (100), 258 (84), 302 (83), 348 (98), 537 (5), 639 (16)

oven temperature programmed from 40°C to 270°C at 10°C min^{-1}. Mass spectra were scanned over *m/z* 200–720 (Instrument 1) and *m/z* 90–650 (Instrument 2). Acetone vapor introduced into the ion source ionization chamber was used as a reagent gas for APPhCI (Instrument 1). The concentration of acetone vapor in a carrier gas (helium) was 10^{-6} g cm^{-3}. The derivatives under investigation were separated on identical quartz capillary columns (30 m × 0.32 mm × 0.25 µm) with a cross-bonded SE-54 stationary phase. The carrier gas flow was 1.5 mL min^{-1} and the helium make-up flow was 15 mL min^{-1}. The sample volume injected into both GCs was 1 µL.

Compounds

All the amino acids used in the study (L-alanine, glycine, L-valine, L-leucine, L-isoleucine, L-proline, L-asparagine, L-methionine, L-arginine, L-histidine, L-threonine, L-serine, L-phenylalanine, L-lysine, L-tryptophan, L-tyrosine and L-cystine) were obtained from Sigma (St. Louis, MO, USA). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Regis Technologies Inc. (Morton Grove, IL, USA) and acetonitrile was purchased from Lecbiopharm (Moscow, Russia).

Amino acid solutions and derivatization procedure

A standard solution of the amino acids used for silylation was prepared in 0.1 M HCl at a concentration of approximately 10^{-5} g mL^{-3}. 100 µL of this solution was evaporated to dryness in a gentle nitrogen stream at room temperature, and then 100 µL of acetonitrile and 100 µL of MTBSTFA were added to the residue. After 30 s of sonification, the mixture was heated at 70°C for 30 min and 1 µL of the cooled reaction mixture was then injected into the GCs. A diluted solution of the reaction mixture (approx. 10^{-11} g injected) was used for estimation of detection limits.

Results and discussion

Seventeen of the most prevalent amino acids in living organisms were chosen as model compounds in this study. The mass spectral data obtained for the TBDMS derivatives of the amino acids in the EI mode are presented in Table 1. As is seen from Table 1, the TBDMS derivatives of the amino acids underwent a considerable fragmentation in the EI mode. The molecular ion is absent for all of the derivatives except Lys.

Table 2. APPhCI mass spectra of the TBDMS derivatives of amino acids.

Derivative	Molecular weight	*m/z* (I, %)
Ala (2TBDMS)	317	317 $[M]^+$(10), 318 $[M+H]^+$ (100)
Gly (2TBDMS)	303	303 $[M]^+$(25), 304 $[M+H]^+$ (100)
Val (2TBDMS)	345	345 $[M]^+$(10), 346 $[M+H]^+$ (100)
Leu (2TBDMS)	359	359 $[M]^+$(20), 360 $[M+H]^+$ (100)
Ile (2TBDMS)	359	359 $[M]^+$(15), 360 $[M+H]^+$ (100)
Pro (2TBDMS)	343	343 $[M]^+$(10), 344 $[M+H]^+$ (100)
Asn (3TBDMS)	474	474 $[M]^+$(30), 475 $[M+H]^+$ (100)
Met (2TBDMS)	377	377 $[M]^+$(10), 378 $[M+H]^+$ (100)
Ser (3TBDMS)	447	447 $[M]^+$(20), 448 $[M+H]^+$ (100)
Thr (3TBDMS)	461	461 $[M]^+$(30), 462 $[M+H]^+$ (100)
Phe (2TBDMS)	393	393 $[M]^+$(20), 394 $[M+H]^+$ (100)
Lys (3TBDMS)	488	488 $[M]^+$(30), 489 $[M+H]^+$ (100)
Arg (4TBDMS)	630	500 $[M–TBDMS-NH]^+$ (100)
His (3TBDMS)	497	497 $[M]^+$(30), 498 $[M+H]^+$ (100)
Tyr (3TBDMS)	523	523 $[M]^+$(40), 524 $[M+H]^+$ (100)
Trp (2TBDMS)	432	432 $[M]^+$(20), 433 $[M+H]^+$ (100)
Cys (4TBDMS)	696	696 $[M]^+$(90), 697 $[M+H]^+$ (100)

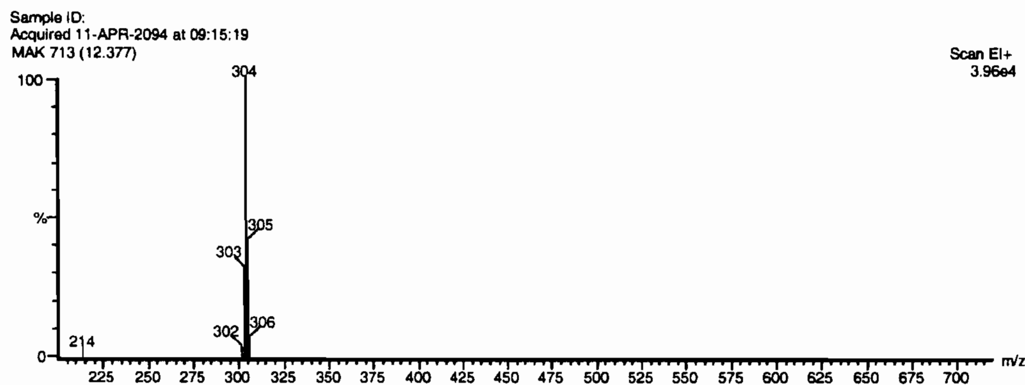

Figure 1. The APPhCl mass spectrum of *N*-*tert*-butyldimethylsilyl glycine *tert*-butyldimethylsilyl ester.

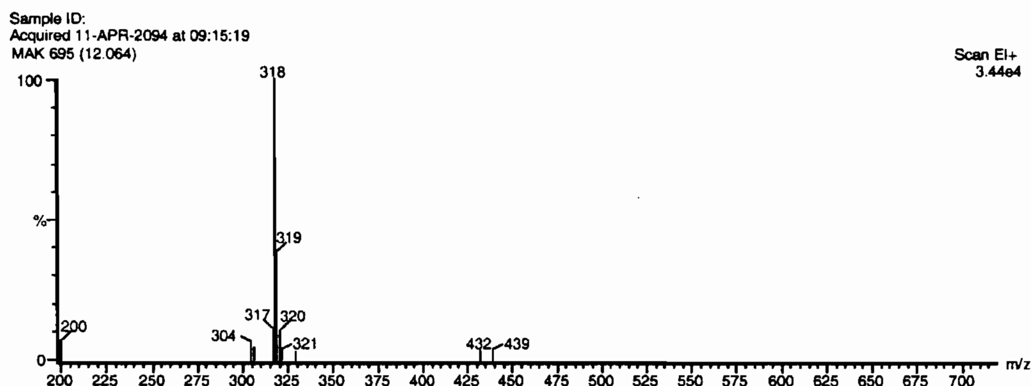

Figure 2. The APPhCl mass spectrum of *N*-*tert*-butyldimethylsilyl alanine *tert*-butyldimethylsilyl ester.

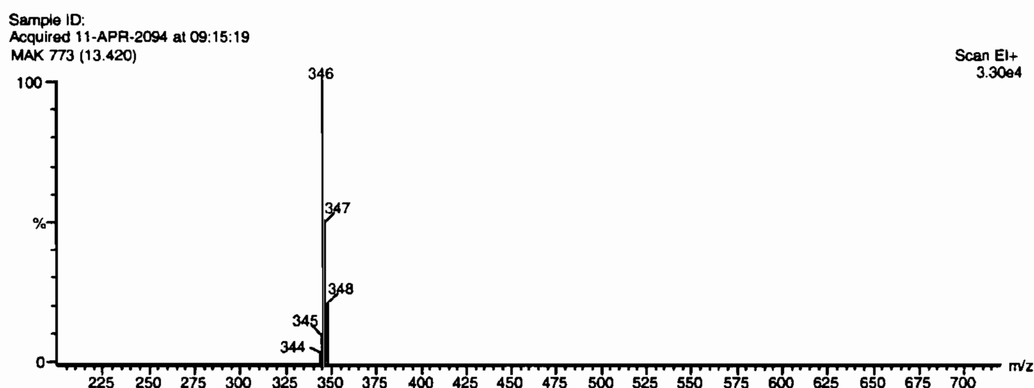

Figure 3. The APPhCl mass spectrum of *N*-*tert*-butyldimethylsilyl valine *tert*-butyldimethylsilyl ester.

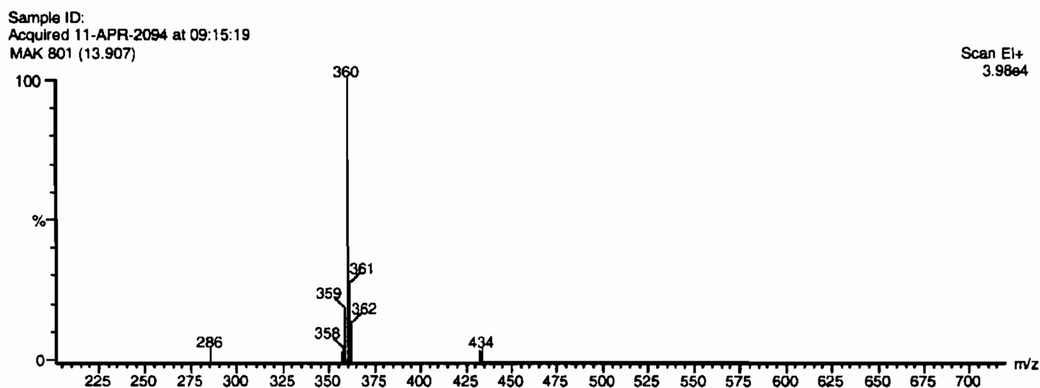

Figure 4. The APPhCl mass spectrum of *N*-*tert*-butyldimethylsilyl leucine *tert*-butyldimethylsilyl ester.

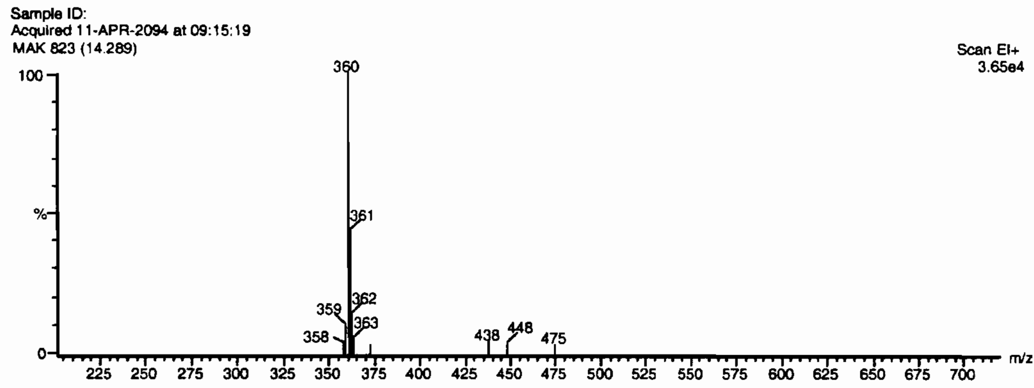

Figure 5. The APPhCI mass spectrum of *N-tert*-butyldimethylsilyl isoleucine *tert*-butyldimethylsilyl ester.

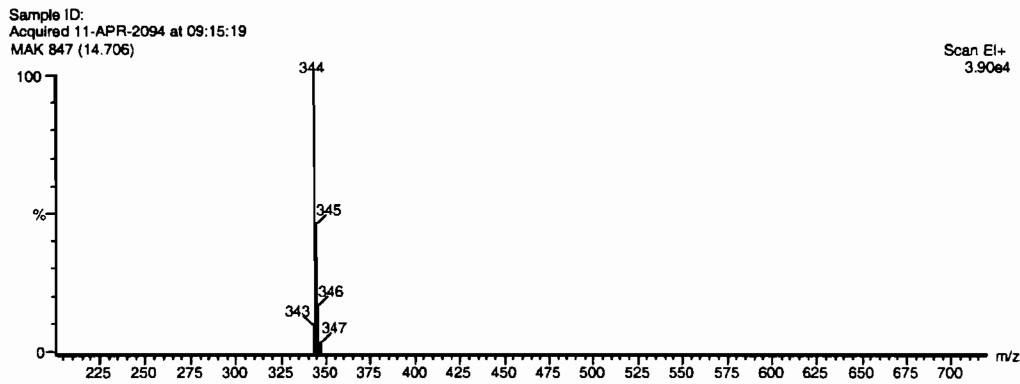

Figure 6. The APPhCI mass spectrum of *N-tert*-butyldimethylsilyl proline *tert*-butyldimethylsilyl ester.

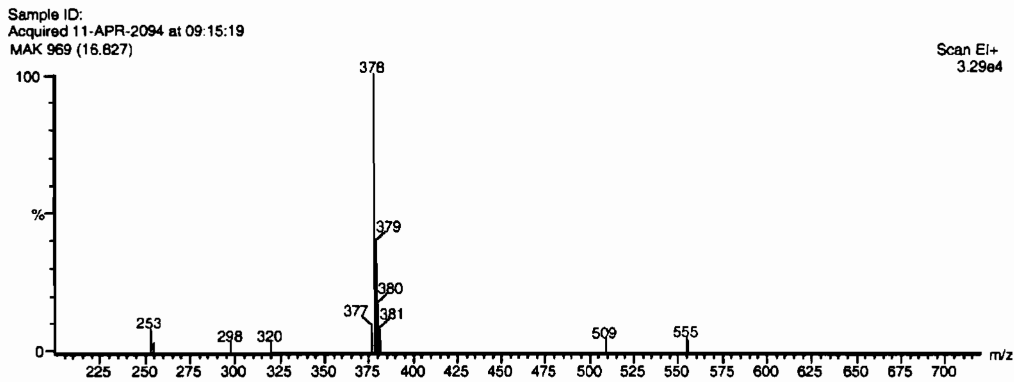

Figure 7. The APPhCI mass spectrum of *N-tert*-butyldimethylsilyl methionine *tert*-butyldimethylsilyl ester.

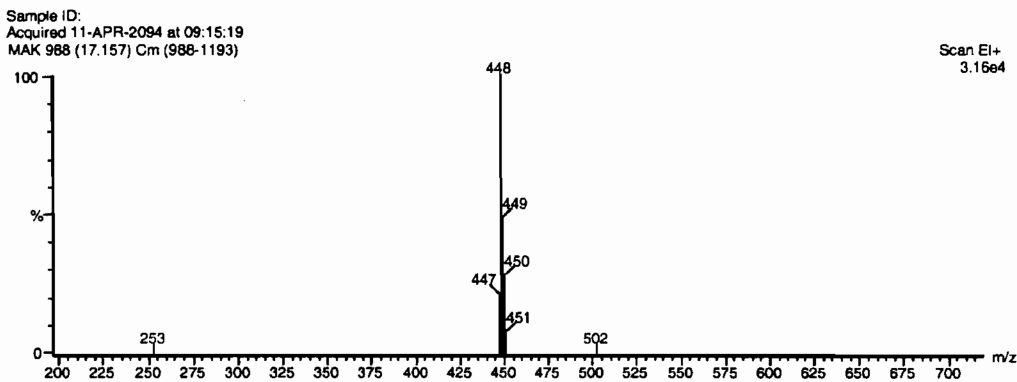

Figure 8. The APPhCI mass spectrum of *N,O*-bis(*tert*-butyldimethylsilyl) serine *tert*-butyldimethylsilyl ester.

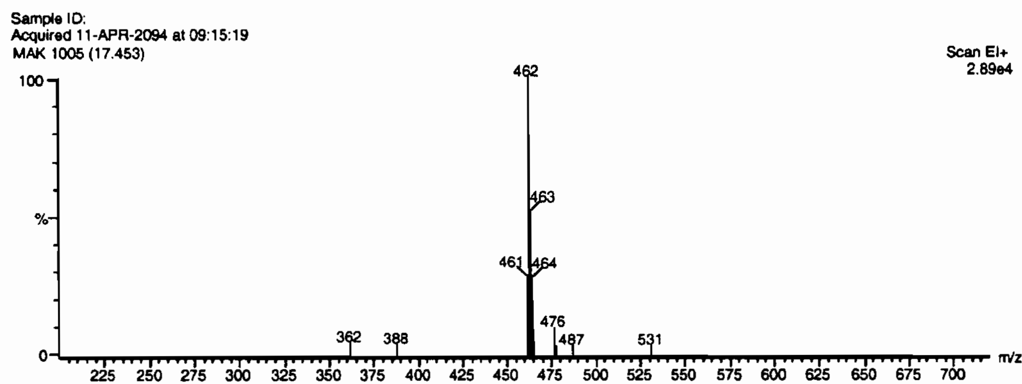

Figure 9. The APPhCI mass spectrum of *N,O*-bis(*tert*-butyldimethylsilyl) threonine *tert*-butyldimethylsilyl ester.

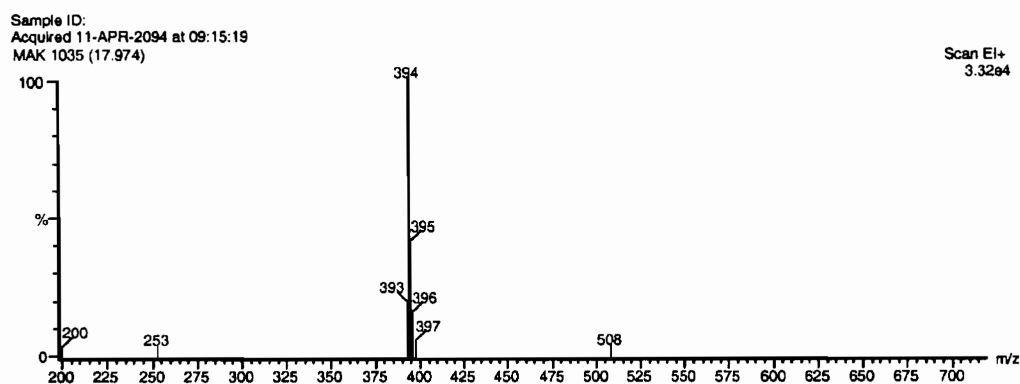

Figure 10. The APPhCI mass spectrum of *N*-*tert*-butyldimethylsilyl phenylalanine *tert*-butyldimethylsilyl ester.

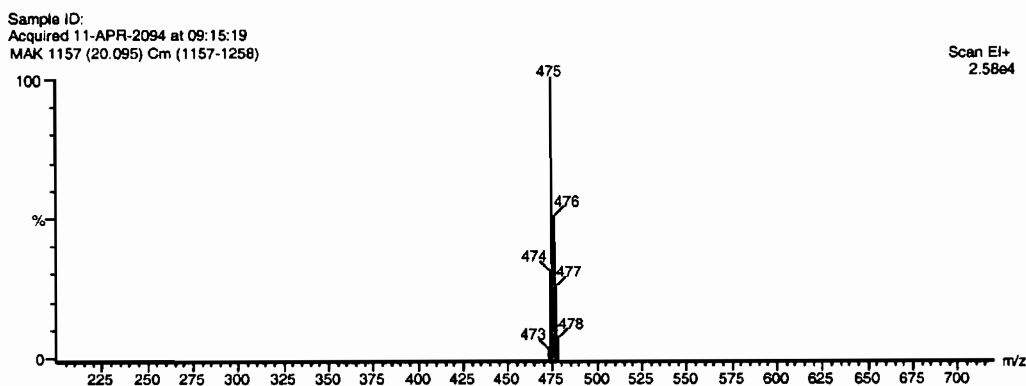

Figure 11. The APPhCI mass spectrum of *N,N'*-bis(*tert*-butyldimethylsilyl) asparagine *tert*-butyldimethylsilyl ester.

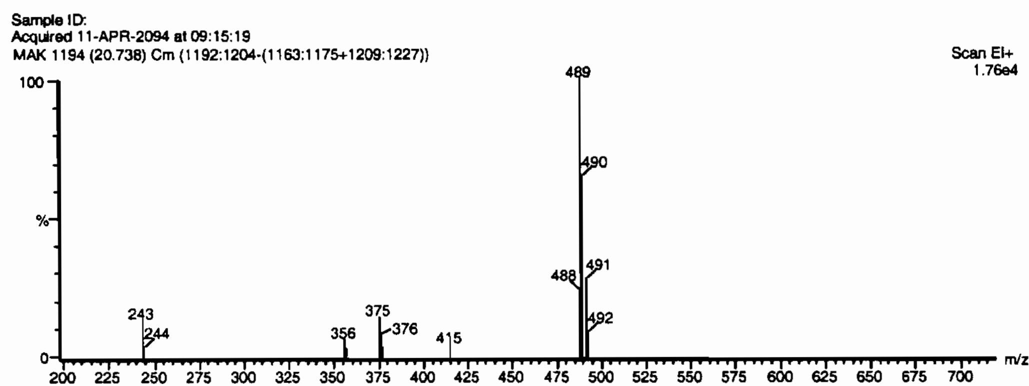

Figure 12. The APPhCI mass spectrum of *N,N'*-bis(*tert*-butyldimethylsilyl) lysine *tert*-butyldimethylsilyl ester.

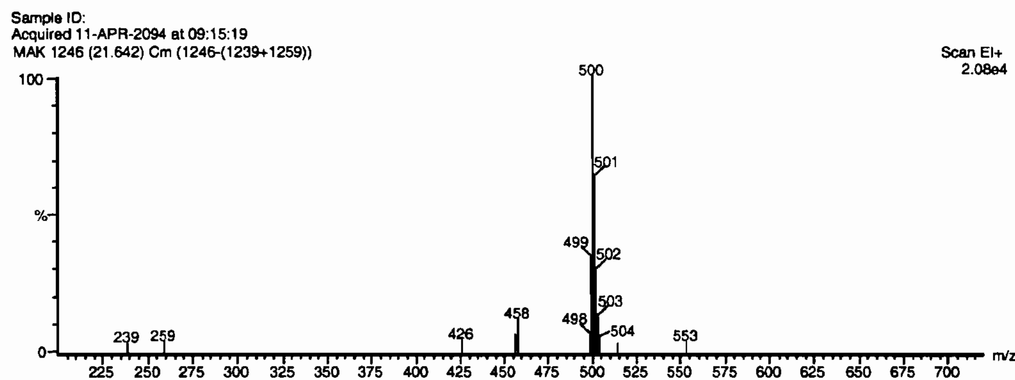

Figure 13. The APPhCI mass spectrum of *N,N'*-bis(*tert*-butyldimethylsilyl) arginine bis(*tert*-butyldimethylsilyl) ester.

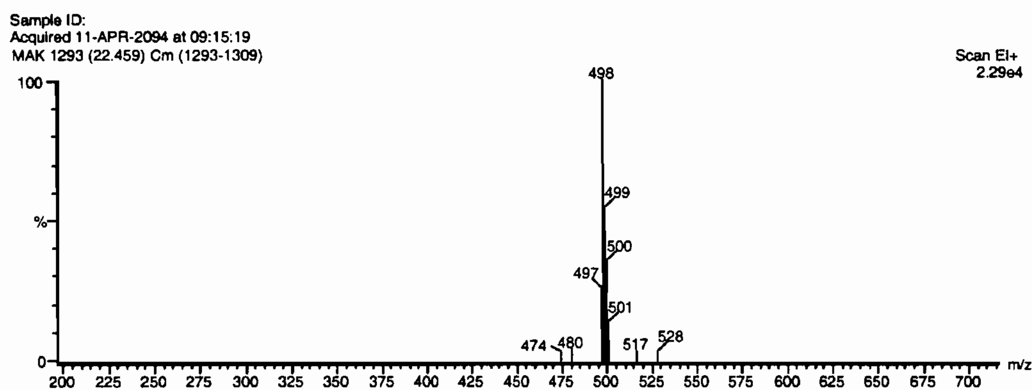

Figure 14. The APPhCI mass spectrum of *N,N*1-bis(*tert*-butyldimethylsilyl) histidine *tert*-butyldimethylsilyl ester.

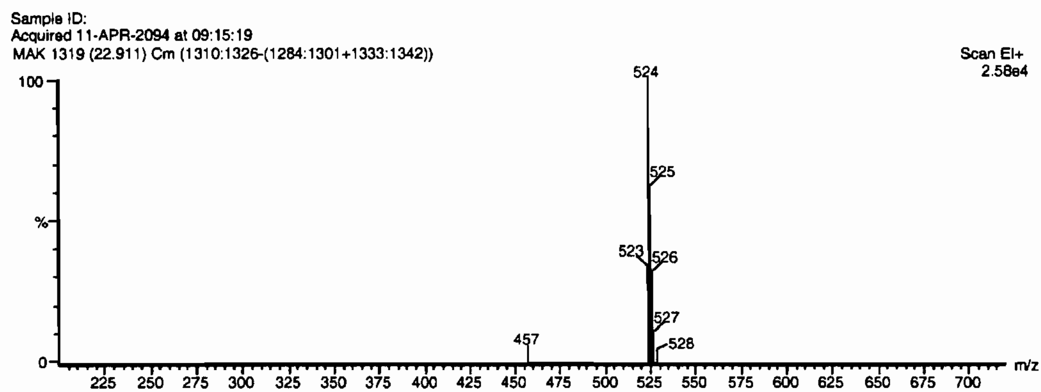

Figure 15. The APPhCI mass spectrum of *N,O*-bis(*tert*-butyldimethylsilyl) tyrosine *tert*-butyldimethylsilyl ester.

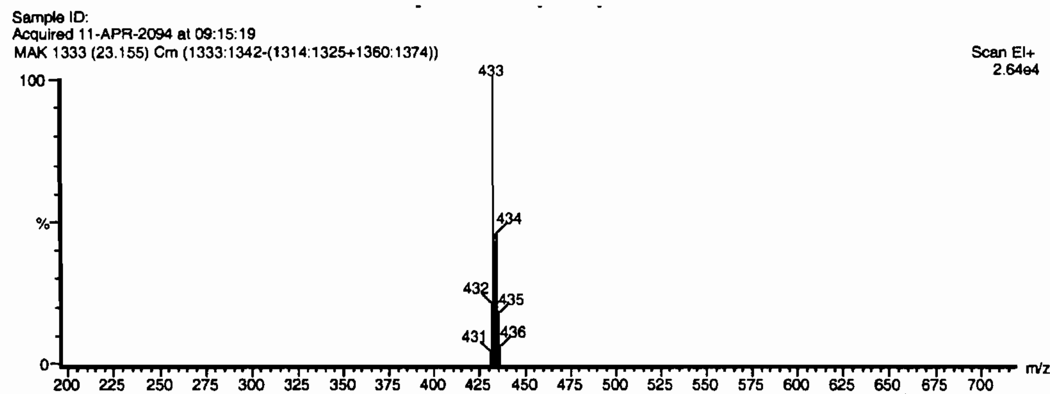

Figure 16. The APPhCI mass spectrum of *N*-*tert*-butyldimethylsilyl tryptophan *tert*-butyldimethylsilyl ester (the TBDMS group is attached to the NH_2 nitrogen).

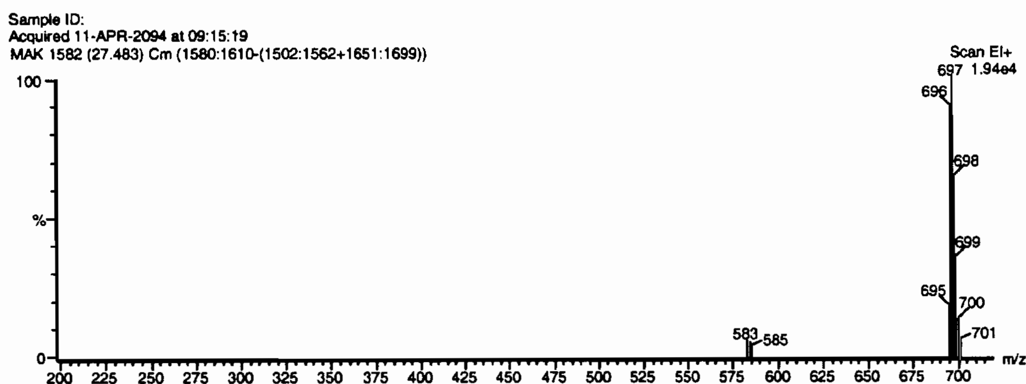

Figure 17. The APPhCI mass spectrum of *N,N'*-bis(*tert*-butyldimethylsilyl) cystine bis(*tert*-butyldimethylsilyl) ester.

The data from atmospheric pressure photochemical ionization mass spectra of the investigated compounds are listed in Table 2. Respective APPhCI mass spectra are presented in Figures 1 to 17. Table 2 and the presented mass spectra demonstrate that the APPhCI mass spectra of the TBDMS derivatives of all the investigated amino acids (except Arg, which features the elimination of a TBDMS–NH– group) consisted of only the molecular and quasimolecular ion peaks and related isotopic ion peaks.

Calculated detection limits for the investigated amino acids derivatives in the SIM mode for EI and APPhCI were 10^{-11}–10^{-12} g μL^{-1} and 10^{-12}–10^{-13} g μL^{-1}, respectively, depending on the compound chosen. To compare APPhCI GC-MS and PICI GC-MS for this particular application, the PICI mass spectra (methane as reagent gas) of the respective TBDMS derivatives reported in Reference 4 are summarized in Table 3. As is seen from Table 3, PICI, unlike APPhCI, results in fragmentation. In addition, the peaks corresponding to the molecular or quasimolecular ions for most of the investigated compounds were of low intensity or not registered at all.

Conclusion

Contrary to EI, APPhCI mass spectra for all but one target amino acid derivative were shown to consist of only molecular and quasimolecular ion peaks. The detection limits for APPhCI GC-MS were slightly better than those for EI GC-MS in the SIM mode: 10^{-12}–10^{-13} g μL^{-1} depending on the amino acid. The reliability of identification of complex

Table 3. PICI mass spectra of the TBDMS derivatives of amino acids.[4]

Derivative	Molecular weight	*m/z* (I, %)
Ala (2TBDMS)	317	318 (100), 302 (90), 260 (72), 232 (100), 158 (14)
Gly (2TBDMS)	303	304 (100), 288 (82), 246 (64), 218 (10), 190 (12)
Val (2TBDMS)	345	346 (100), 330 (96), 288 (77), 186 (28)
Leu (2TBDMS)	359	360 (95), 344 (83), 302 (100), 274 (11), 246 (11), 200 (33)
Pro (2TBDMS)	343	344 (96), 328 (86), 286 (94), 258 (13), 230 (15), 184 (100)
Asn (3TBDMS)	474	475 (33), 459 (45), 158 (21)
Met (2TBDMS)	377	378 (100), 362 (49), 330 (13), 320 (45), 264 (11), 218 (12)
Ser (3TBDMS)	447	432 (100), 390 (92), 334 (13), 288 (10)
Thr (3TBDMS)	461	462 (59), 447 (100), 404 (80), 348 (11), 302 (21)
Phe (2TBDMS)	393	394 (100), 378 (86), 336 (74), 280 (16), 243 (19), 120 (14)
Lys (3TBDMS)	488	489 (72), 473 (49), 431 (48)
Arg (4TBDMS)	630	500 (43), 484 (35), 422 (38), 341 (24), 328 (18)
His (3TBDMS)	497	498 (65), 482 (58), 441 (63)
Tyr (3TBDMS)	523	525 (63), 509 (59), 466 (63)

mixture components and the selectivity of their quantitation should be higher when APPhCI GC-MS is used.

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