AD-A162 481 COMPLEX MIXTURE ANALYSIS BY PHOTOIONIZATION MASS SPECTROMETRY WITH A VUV H... (U) ARIZONA UNIV TUCSON DEPT OF CHEMISTRY T C HUTH ET AL. 06 DEC 85 TR-44 1/1

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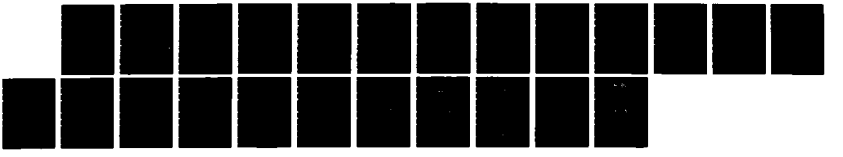

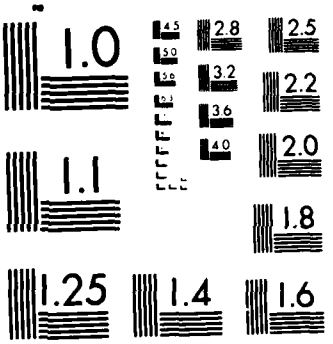

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Trace organic analysis in complex matrix presents one of the most challenging problems in analytical mass spectrometry. When ionization is accomplished non-selectively using electron impact, extensive sample clean-up is often necessary in order to isolate the analyte from the matrix. Sample preparation can be greatly reduced when the VUV H_2 laser is used to selectively photoionize only a small fraction of compounds introduced into the ion source. This device produces parent ions only for all compounds whose ionization potentials lie below a threshold value determined by the photon energy of 7.8 eV. (Continued on other side)

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Results are presented which indicate the ability of this ion source to discriminate against interfering matrix components, in simple extracts from a variety of complex "real world" matrices, such as brewed coffee, beer, and urine.

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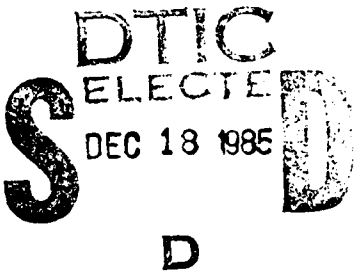

Department of Chemistry
University of Arizona
Tucson, Arizona 85721

December 6, 1985

COMPLEX MIXTURE ANALYSIS BY PHOTOIONIZATION MASS SPECTROMETRY WITH A VUV HYDROGEN LASER SOURCE

Thomas C. Huth and M. Bonner Denton
Department of Chemistry
University of Arizona
Tucson, Arizona 85721

Abstract

Trace organic analysis in complex matrix presents one of the most challenging problems in analytical mass spectrometry. When ionization is accomplished nonselectively using electron impact, extensive sample clean-up is often necessary in order to isolate the analyte from the matrix. Sample preparation can be greatly reduced when the VUV H_2 laser is used to selectively photoionize only a small fraction of compounds introduced into the ion source. This device produces parent ions only for all compounds whose ionization potentials lie below a threshold value determined by the photon energy of 7.8 eV.

The only observed interference arises from electron impact ionization, when scattered laser radiation interacts with metal surfaces, producing electrons which are then accelerated by potential fields inside the source. These can be suppressed to levels acceptable for practical analysis through proper instrumental design.

Results are presented which indicate the ability of this ion source to discriminate against interfering matrix components, in simple extracts from a variety of complex "real world" matrices, such as brewed coffee, beer, and urine.

Complex Mixture Analysis by Photoionization Mass Spectrometry with a Vacuum Ultraviolet Hydrogen Laser Source

INTRODUCTION

The need for selective techniques in mass spectrometric analysis of complex mixtures has been well documented [1]. Mass spectrometry is one of the most sensitive techniques available for trace analysis, but for typical analytical samples, the lack of ionization selectivity afforded by common ion sources usually necessitates complex and tedious preseparation steps in order to produce interpretable results. The problem is compounded by the extensive fragmentation produced by these sources, resulting in a large number of ion masses for each component present. One approach to achieving selectivity in the ionization process is laser-induced photoionization, which can be accomplished using a variety of strategies, employing resonant and non-resonant single and multiphoton processes [2]. Selective single-photon ionization of compounds from simple mixtures has been demonstrated, using the Lyman band output from a molecular hydrogen laser [3]. Ions produced by this process consist of parent molecular ions only. Species whose ionization potentials lie above the radiation energy of 7.8 eV are completely rejected. Among the types of compounds ionized below this threshold are many amines and nitrogen heterocycles, a class which encompases a large number of pharmacologically-active molecules.

The aim of this study is to demonstrate the capabilities of H_2 laser photoionization mass spectrometry for selective analysis in real-world matrices. Of particular interest is its applicability in the area of rapid

screening of drugs and drug metabolites in biological samples. Reduction of non-selective ionization by electron impact is discussed. This was established in previous work as the most important interference for simple mixtures [3]. It results from acceleration of stray electrons produced in the ion source, primarily by interaction of scattered laser radiation with metal surfaces. Spectra obtained from extracts of spiked beer, coffee, soy sauce, urine, and blood serum are shown. The food matrices were chosen as models for this work because they are well-characterized, readily available, and are known to contain nitrogen compounds of the type suitable for ionization by the H_2 laser.

EXPERIMENTAL

Instrumentation: The H_2 laser photoionization mass spectrometer (H_2 LPMS) system employed in this study has been described previously [3,4].

For this work, the electrostatic multiplier was replaced with a Galileo Electro-Optics M-306 magnetic electron multiplier (Galileo Electro-Optics Corp., Galileo Park, Sturbridge, MA 01518), operated at a dynode voltage between -1500 and -1950V. The spectra in Figures 1 and 2 were acquired using a specially developed wide-bandwidth high-gain electrometer preamplifier. This device amplifies the multiplier output current pulses with a voltage/current characteristic of 5×10^6 V/A at a bandwidth greater that 50 MHz. At the signal levels of the present experiment, its output is on the order of 1V.

In order to introduce solid extraction residues, a direct probe inlet was constructed using a 22.9 cm length of 6.4 mm O.D. stainless steel

tubing. This probe rod was inserted into the ion source through a ball valve vacuum lock, to which the rod was attached using a Cajon Ultra-Torr vacuum fitting (Cajon Co., 9760 Shepard Rd., Macedonia, OH 44056). A sample vial at the end of the probe rod was heated using 1.0 A of direct current through a 10 cm length of 30 gauge Nichrome wire, wound around the vial. Leads for heating of the filament were threaded through the center of the rod, and sealed at the top using a drop of high-vacuum epoxy. The atmosphere side of the ball valve was also fitted with a sidearm for roughing after attachment of the rod. The ion source was held at 80 degrees C during the acquisition of all spectra reported here.

For the electron impact experiments, liquids and solutions were injected through the heated inlet system described previously [3], also operated at 80 degrees C.

Several modifications were made with the aim of reducing photoelectron production from metal surfaces in the ion source. A 6.4 mm diameter circular aperture was installed at the point of entry of laser radiation into the source housing, to restrict the divergent beam. The aperture was placed on the laser side of the CaF_2 lens which seals the ion source vacuum. A metal sleeve attached to the direct probe inlet aperture prevents acceleration of thermionic electrons from the hot filament. The bottom of the sleeve is covered with a layer of fine tungsten mesh grid material. Finally, an angled exit window assembly directs reflected laser radiation downward, in order that the formation of electrons occurs at a location remote from the accelerating fields. Since the laser beam is not polarized, the exact angle of the window is not critical, so long as it is less than Brewster's angle [5].

The window assembly used in these experiments has an angle of 45 degrees. A collector electrode was installed inside the window mount, but was observed to have no effect on the magnitude of electron impact signals at applied voltages from zero up to the accelerating voltage of +500 V, and was therefore not used in these experiments.

Spectra were acquired by photographing the oscilloscope trace using Polaroid Type 47 film (ASA 3000).

Materials: For the extraction of complex matrix samples, ACS Reagent grade dichloromethane and Spectrophotometric grade n-heptane were used as received. Reagent grade isoamyl alcohol was purified by redistillation.

Coffee, soy sauce, and an American lager beer were purchased directly from the grocery store shelf as complex matrix models. The coffee was drip-brewed fresh for each run according to manufacturer's instructions, and separated immediately thereafter for the analysis. The soy sauce and beer were used directly from their containers. Soy sauce was stored at room temperature, while beer was stored at 2 degrees C, a fresh sample being used for each run.

The urine was a pooled sample collected from seven volunteers, and stored in a polyethylene bottle at 2 degrees C. Total storage time over the course of these experiments was approximately 2 weeks.

Lyophilized human control serum (Ortho Diagnostics, Inc., Raritan, NJ) was reconstituted fresh for each run. The dry sample was stored at 2 degrees C.

Reagent grade 4-methoxyaniline was recrystallized from hot H_2O, and thereafter stored at 2 degrees C in the dark. Stock solutions of 1 mg/mL

were prepared fresh every 2 days in 0.1 M HCl, and were also stored between runs at 2 degrees C in the dark.

Samples of the phenothiazine tranquillizers chlorpromazine HCl and trimeprazine tartarate (Smith, Kline and French Laboratories, Philadelphia, PA), and promethazine HCl (Wyeth Laboratories, Malvern, PA) were supplied by the manufacturers. Stock solutions of 5 µg/mL were prepared fresh every 7 days, and were stored at 2 degrees C in amber glass bottles.

Sample Preparation: For the food matrices, a basic extraction was performed using 10 mL of sample, spiked at the 50 ppb level with 4-methoxyaniline. This was placed into a glass centrifuge tube and made basic to pH 11 using 1M NaOH. The solution was then extracted using 10 mL dichloromethane, by mechanical shaking for 20 minutes. The mixture was centrifuged, and the organic layer transferred by suction to a clean tube. This was back-extracted with 10 mL 0.1 M HCl, in the same manner. The acid phase was again separated and transferred to a clean tube, made to pH 11, and re-extracted with another 10 mL dichloromethane. The final organic extract containing the basic fraction of the sample was removed into a tapered glass tube and evaporated to approximately 150 µL, in a 60 degree C water bath under a stream of N_2. The solution was then transferred into a direct probe sample vial, and evaporated to dryness in the water bath. The photoionization mass spectrum of the residue was determined immediately.

Since it has been reported that free base phenothiazines are adsorbed strongly on glass surfaces [6], polycarbonate centrifuge tubes were used for the extraction of these drugs from urine and serum. For these samples, a single extraction was performed on a 10 mL aliquot, spiked at 100 ppb of the

drugs. The sample, made basic to pH 11 with 1M NaOH, was extracted once with n-heptane/1.5% isoamyl alcohol, by mechanical shaking for 20 minutes. Since the phenothiazines are light-sensitive [6], especially in their free base form, the extraction tubes were covered with aluminum foil during the shaking period to exclude room light. The mixture was centrifuged, and the organic layer separated by suction. This was evaporated on a 60 degree C water bath, under a stream of N_2, to 150 µL. After transfer to a sample vial, the solution was evaporated to dryness under vacuum at room temperature, and the spectrum determined immediately.

Diphenylamine was used as a mass marker in these experiments, and was added to sample vials just prior to running of the spectra.

RESULTS AND DISCUSSION

Electron impact interference: In initial experiments with this system [3], it quickly became clear that a major source of interference in the analysis of mixtures was electron impact ionization caused by stray electrons in the ion source. For low-level analysis, it is crucial that interaction of the VUV laser beam with metal surfaces inside the ion source be minimized. Following the instrumental modifications outlined above, this interference has been reduced to the level shown in Figure 1. The intensity of the $CHCl_2^+$ feature produced by electron impact for a 1 µL injection of chloroform is at approximately the level of photoion signal for 50 ng of 4-aminotoluene.

The fragmentation observed for electron impact signals in these spectra indicates that much of this ionization is produced by low-energy electrons. The fragment ion feature observed near 50 amu in the 70 eV electron impact spectrum of benzene is also present here, but at greatly reduced intensity relative to the molecular ion (Figure 2a). This is consistent with the proposition that the electrons effective in producing detectable ions are actually secondaries sputtered from the source electrodes [7]. As the level of primary formation via the laser scattering mechanism is reduced, the weak fragment features disappear below the detection limit, and only the intense molecular ion remains (Figure 2b). At the reduced levels, spectra of mixtures become cleaner, but it also becomes more difficult to distinguish signals produced by electron impact from photoions by simple inspection. This distinction can be made easily through adjustment of ion source parameters [7].

Complex mixture analysis: The photoion mass spectra of basic extracts of spiked beer, coffee, and soy sauce are shown in Figure 3, along with a list of ion masses in Table 1.

Many compounds ionized below the H_2 laser photon energy of 7.8 eV are amines and N-heterocycles [3], which will be concentrated into the basic fraction if they are present. Basic fractions of food extracts consist mainly of heterocyclic nitrogen compounds such as pyrazines and pyridines [8]. For coffee, a number of pyrroles, indoles, and quinolines are also present [10]. In all, over 100 compounds have been identified in this fraction for coffee [9-11], and about 35 each for beer [12] and soy sauce [13]. Selective photoionization greatly simplifies the mass spectra of these

mixtures to a few signals. Although it is clear in each case that several matrix compounds are ionized, the lack of fragmentation prevents this fact from greatly confusing the spectra. It should be noted here that fragmentation can also be suppressed using low-energy electron impact, but this is accompanied by a reduction in ionization efficiency, resulting in a substantial loss of sensitivity.

Spectra of all pure compounds and synthetic mixtures obtained thus far by H_2 LPMS consist of parent molecular ions only. The spectra in Figure 4 however, contain several low-mass ions, which are not likely to represent intact molecules. They may be fragments that result from pyrolysis on the hot filament used to heat the direct probe tip. This phenomenon has been reported previously in electron impact experiments [14,15]. The possibility that they arise from a two-photon process cannot be discounted, although the probability of such an event is smaller than that for single-photon ionization by at least 5 orders of magnitude under the conditions of this experiment. This would imply a very large excess of the precursor. The laser radiation at 7.8 eV represents a rather small excess of energy (1 eV or less) over the ionization potentials of even the easiest-ionized of molecules. Under these conditions, dissociative ionization proceeding through autoionizing states of the neutral molecule, when energetically possible, would not be expected to produce fragments much smaller than the molecuiar ion [16]. These signals do not appear to arise from stray electron impact, since their intensity is not affected by the hardware changes described above.

Electron impact signals that do remain in these spectra appear as a weak, diffuse background. They are in general barely detectable on the photographs.

The spectra of simple extracts of urine and blood serum spiked at 100 ppb with three phenothiazine tranquillizers are shown in Figure 4, with ion masses listed in Table 2. Although the relationship between blood level of these drugs and therapeutic effect has not as yet been clearly defined [17], the level of 100 ppb is well within the estimated "therapeutic window" for chlorpromazine [18]. Excretion of phenothiazines into the urine concentrates them into the parts-per-million range [19]. The photoion mass spectra of these extracts are remarkably clear, with only a few significant matrix signals observed.

Vacuum ultraviolet H_2 laser photoionization produces greatly simplified mass spectra for complex mixtures, because of the selectivity of the threshold photoionization process and the lack of fragmentation.

The instrumentation required for the H_2 LPMS experiment is simple and inexpensive to build and operate. Speed of analysis is enhanced by the ability to introduce samples directly by solution injection. Sensitivity is fully adequate for determination of at least one class of easily-ionized drug compounds at physiologically meaningful levels. Use of a transient recorder for signal averaging should result in further sensitivity enhancement. Based on the results reported here, and the fact that many pharmaceuticals and drugs of abuse contain the functionalities associated with easy ionizability, rapid trace level drug screening by this technique appears to be feasible. Further studies that bear on this question are currently in progress, including evaluation of detection limits using signal

averaging, and determination of the ionizability of a wider range of drugs of interest.

ACKNOWLEDGMENT

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REFERENCES

1. K. Beyermann in "Applications of Mass Spectrometry to Trace Analysis", S. Facchetti, Ed., Elsevier, Amsterdam, 1982, p. 1-67, and references therein.

2. R. J. Cotter, Anal. Chem. 56 (1984), 485A.

3. T. C. Huth and M. B. Denton, Int. J. Mass Spectrom. Ion Phys. 67 (1985), 199.

4. J. S. Babis, T. C. Huth and M. B. Denton, Rev. Sci. Instrum. 56 (1985) 1969.

5. F. A. Jenkins and H. E. White, "Fundamentals of Optics", 4th Ed., McGraw-Hill, New York, 1976.

6. S. H. Curry, Anal. Chem., 40 (1968), 1251.

7. T. C. Huth and M. B. Denton, manuscript in preparation.

8. J. A. Maga and C. E. Sizer, CRC Critical Reviews in Food Technology, 4 (1973), p. 57.

9. O. G. Vitzthum and P. Werkhoff, J. Food Sci., 39 (1974), 1210.

10. O. G. Vitzthum and P. Werkhoff, Z. Lebensm. Unters. Forsch., 156 (1974), 300.

11. O. G. Viththum and P. Werkhoff, J. Agric. Food Chem., 23 (1975), 516.

12. R. J. Harding, H. E. Nursten, and J. J. Wren, J. Sci. Fd. Agric., 28 (1977), 225.

13. N. Nunomura, M. Sasaki, Y. Asao, and T. Yokotsuka, Agric. Biol. Chem., 42 (1978), 2123.

14. A. G. Harrison in "Mass Spectrometry of Organic Ions", F. W. McLafferty, Ed., Academic Press, New York, 1963, p. 21.

15. C. E. Brion, Anal. Chem. 37 (1965) 1706.

16. J. Berkowitz, "Photoabsorption, Photoionization, and Photoelectron Spectroscopy", Academic Press, New York, 1979.

17. J. G. B. M. Noten and D. R. A. Uges in "The Serum Concentration of Drugs", F. W. H. M. Merkus, Ed., Exerpta Medica, Amsterdam, 1980, p. 126.

18. S. H. Curry, Psychopharmacol. Commun., 2 (1976), 1.

19. J. A. F. deSilva in "GLC and HPLC Determination of Therapeutic Agents", K. Tsuji, Ed., Marcel Dekker, New York, 1978, p. 592

Table 1: Ion Masses for H_2 LPMS of Food Basic Extracts

Matrix	Ion Flight Time (μsec)	Mass (amu)[a]	Origin
Beer	37.1	55	matrix
	54.7	123	4-methoxyaniline spike
	59.0	144	matrix
	62.3	160	matrix
	63.9	169	diphenylamine marker
	66.9	186	matrix
Coffee	36.8	55	matrix
	44.4	82	matrix
	47.1	92	matrix
	54.3	123	4-methoxyaniline spike
	56.2	132	matrix
	59.0	145	matrix
	63.6	169	diphenylamine marker
	73.7	228	matrix
Soy sauce	32.9	43	matrix
	36.9	55	matrix
	37.4	56	matrix
	41.2	69	matrix
	44.5	81	matrix
	47.3	92	matrix
	54.6	123	4-methoxyaniline spike
	56.4	131	matrix
	62.8	165	matrix
	63.6	169	diphenylamine marker

a. Unknown ion masses were calculated with respect to the diphenylamine mass marker using flight times measured directly from the low-resolution spectra shown. The standard deviation of 17 replicate mass determinations made in this manner for the promethazine molecular ion (284.4 amu) was 1.4 amu.

Table 2: Ion Masses for H_2 LPMS of Biological Matrix Extracts

Matrix	Ion Flight Time (μsec)	Mass (amu)[a]	Origin
Urine	63.9	169	diphenylamine marker
	66.9	198	contaminant
	82.4	284	promethazine spike[b]
	84.3	299	trimeprazine spike[b]
	87.3	319	chlorpromazine spike[b]
Serum	48.5	96	matrix
	63.8	169	diphenylamine marker
	72.7	221	matrix
	82.6	284	promethazine spike[b]
	84.3	299	trimeprazine spike[b]
	87.1	319	chlorpromazine spike[b]

a. See note a, Table 1.

b. Isotope structure appears only as a slight broadening on the time scale of these spectra.

FIGURE CAPTIONS

Figure 1: H_2 LPMS of 50 ng 4-aminotoluene (107 amu) injected in 1 µl $CHCl_3$.

Figure 2: Electron impact signals for benzene. 0.5 µl injections; A) before modifications, B) after modifications. Signal at 43.5 usec is the benzene molecular ion feature. Note that in A), this signal is off scale. The actual intensity exceeds that shown by a factor of about 5. Signal at 34 µsec is the fragment ion feature near 50 amu. Diphenylamine mass marker appears at 169 amu.

Figure 3: H_2 LPMS of food basic extracts: A) beer, B) coffee, C) soy sauce. 10 ml samples spiked at 50 ppb 4-methoxyaniline (123 amu) prior to extraction. Diphenylamine mass marker appears at 169 amu.

Figure 4: H_2 LPMS of biological extracts: A) urine, B) serum. 10 ml samples spiked at 100 ppb each promethazine HCl (284 amu), trimeprazine tartarate (299 amu), and chlorpromazine HCl (319 amu) prior to extraction. Diphenylamine mass marker appears at 169 amu.

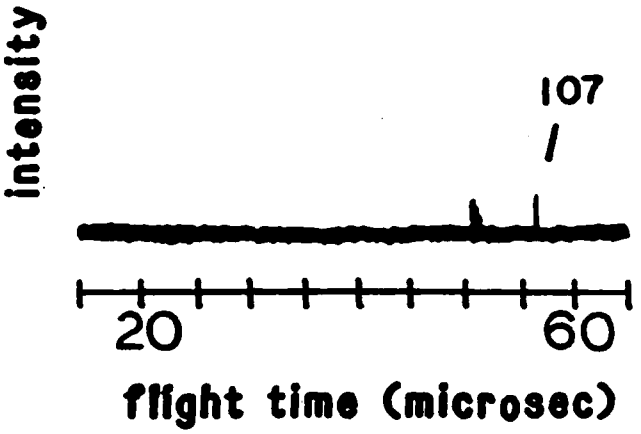


FIGURE 1

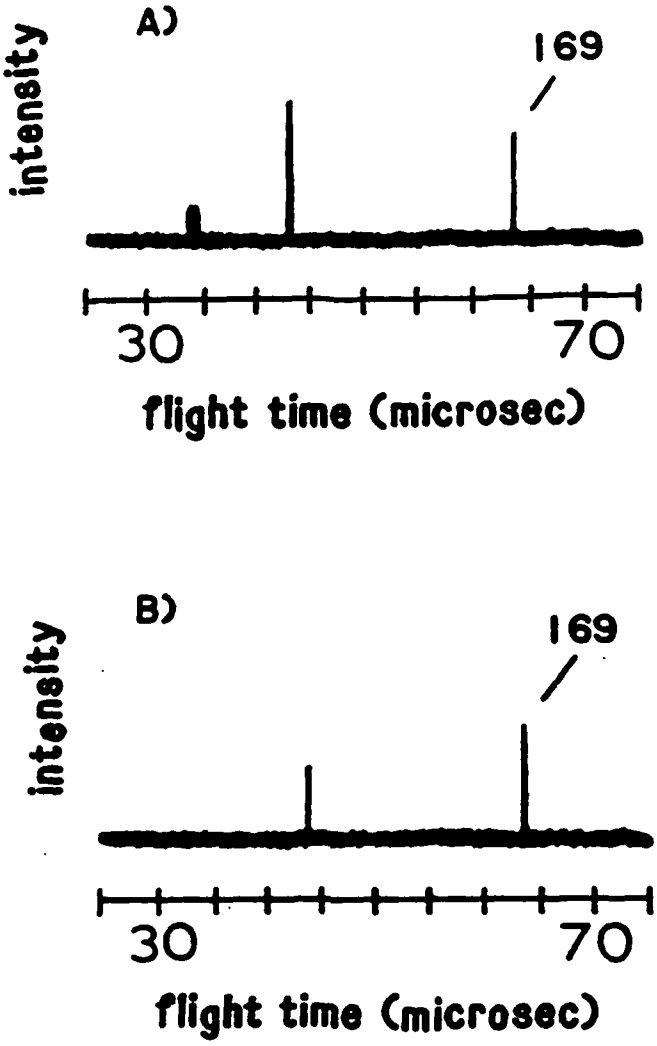
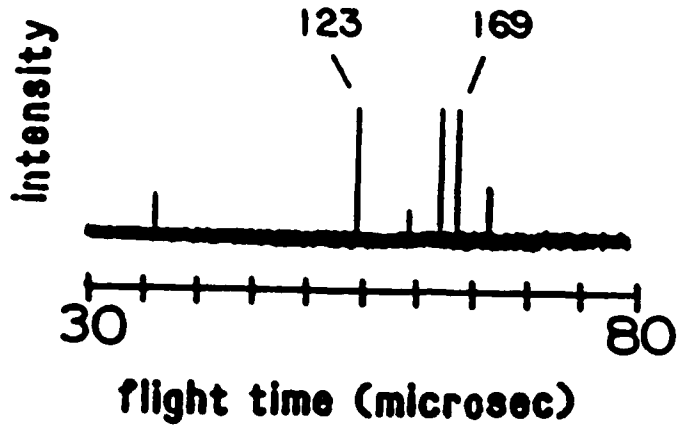
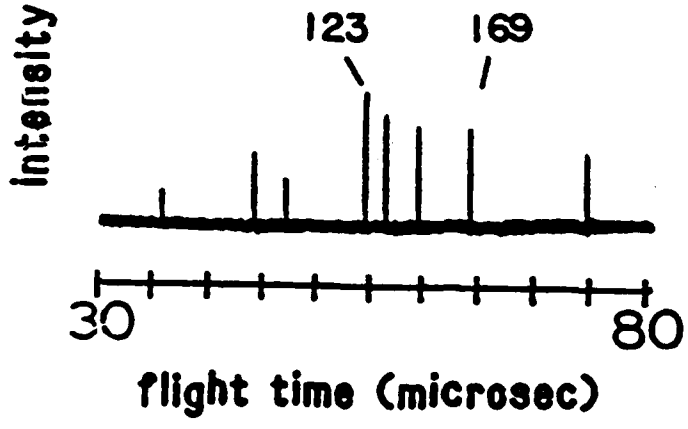

A)
169
intensity
30
70
flight time (microsec)
B)
169
intensity
30
70
flight time (microsec)

FIGURE 2

A) Beer



B) Coffee



C) Soy sauce

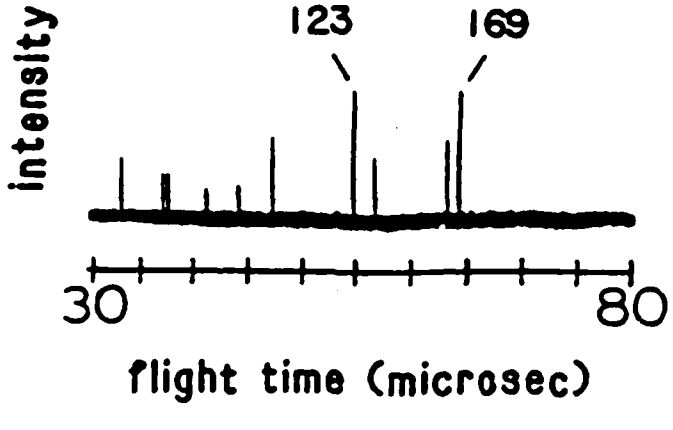
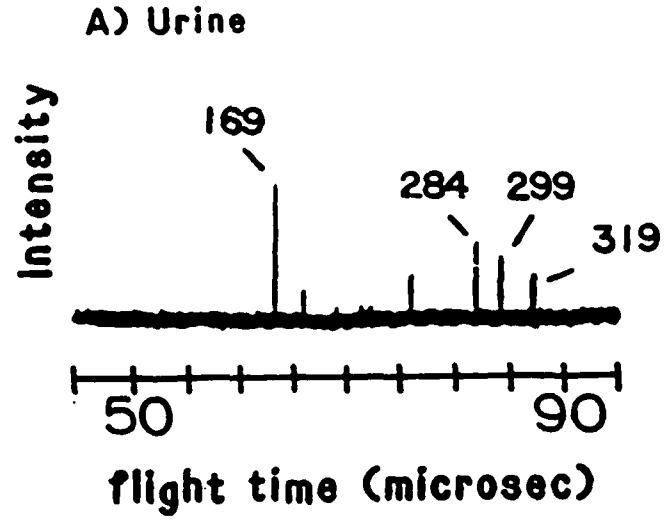


FIGURE 3


A) Urine
Intensity
169
284
299
319
50
90
flight time (microsec)

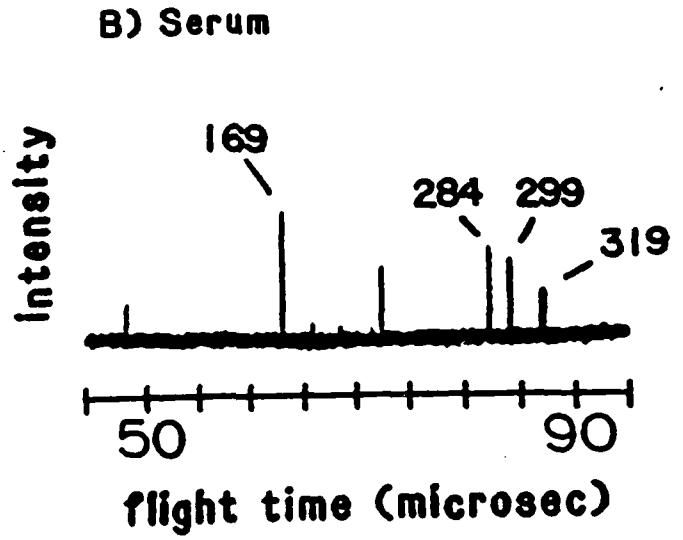

B) Serum
Intensity
169
284
299
319
50
90
flight time (microsec)

FIGURE 4

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