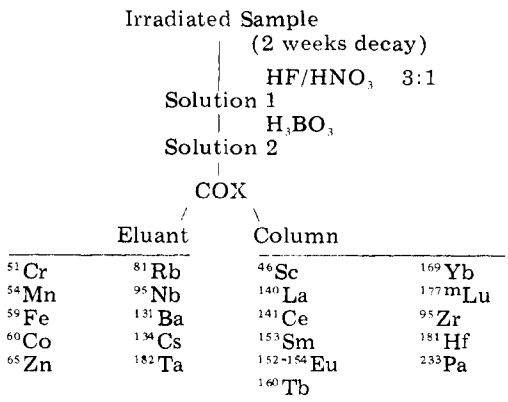

Figure 1. Proposed separation scheme

^{46}Sc, ^{95}Zr, ^{141}Ce, ^{152}Eu, ^{181}Hf, and ^{233}Pa were retained completely; ^{60}Co, ^{82}Rb, ^{95}Nb, ^{131}Cs, and ^{182}Ta passed directly in the eluate; ^{51}Cr and ^{59}Fe were partially adsorbed; ^{54}Mn, retained in the beginning of the elution, began to pass partially in the eluate. Photopeaks of ^{65}Zn (511 keV, 1115 keV) invisible before the removal of rare earth elements became visible in the eluate.

The fact that ^{51}Cr and ^{59}Fe are partially adsorbed prompted us to look for another medium capable of eluting these two ions completely. We tried various concentrations of HCl and H_3BO_3. With HCl, ^{51}Cs and ^{59}Fe are totally eluted. However ^{46}Sc begins to partition and, with saturated H_3BO_3, we obtained satisfactory results.

Afterwards, a modification in the procedure was introduced. Instead of using H_3BO_3 only as eluant, we added saturated H_3BO_3 to the dissolved sample before loading the sample onto the column. This step is useful in cases where there is a precipitate of some insoluble fluoride after dissolution of the sample in HF medium; by formation of fluoride complexes, saturated H_3BO_3 allows the dissolution of that precipitate. This procedure also allows the use of normal vessels instead of Teflon vessels during the elution. Very interesting results were seen: ^{46}Sc, ^{181}Hf, ^{233}Pa and the REE are quantitatively retained whereas other elements pass. The retention of ^{233}Pa on the column allows the resolution of the ^{51}Cr and ^{233}Pa interference near 311 keV. On the other hand, Sc and REE do not impede the gamma spectra of ^{181}Hf or ^{233}Pa, these radioelements being sufficiently active and having some other interference-free photopeaks.

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Vacuum Nebulizing Interface for Direct Coupling of Micro-Liquid Chromatograph and Mass Spectrometer

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There has been an increasing interest in the direct coupling of a liquid chromatograph (LC) with a mass spectrometer (MS) (*1–8*). Among recent developments in this field, are (1) moving wire (*1*) and moving belt (*2*), (2) silicone membrane separator (*3*), (3) atmospheric pressure ionization (*4*), (4) direct introduction of a portion of the column effluent into the CI chamber (*5–7*), and (5) use of a jet separator for GC-MS (*8*). The latter two methods are similar to each other since both utilize the solvent vapor as the reagent gas for CI. However, both still have some disadvantages; in method 4, only a few percent of the effluent from an ordinary separation column is introduced into the ion source, and an uniform evaporation of the effluent is not always attained, while in method 5, relatively involatile compounds are difficult to vaporize effectively with a conventional jet separator for GC-MS.

To overcome these problems, a new vacuum nebulizing interface for LC-MS coupling was developed. With this interface, a more general system for LC-MS direct coupling can be established, where the wide range of flow rate of the solvent can be stably interfaced without any conventional splitting and even fairly involatile compounds can effectively be introduced into the ion source at relatively lower temperatures than those required for nonnebulizing direct introduction. Further, this device is also quite effective for sample introduction of polar and/or large molecules of which mass spectra are otherwise difficult to take (*9*).

EXPERIMENTAL

Nebulizing Interface. A schematic diagram of the newly designed vacuum nebulizing interface is shown in Figure 1. The total effluent from a micro LC ranging between 2 and 16 μL/min was introduced into the nebulizer through a coaxial stainless steel capillary tube A (o.d. of 0.35 mm × i.d. of 0.15 mm with core-wire of 0.13 mm). The coaxial capillary nozzle is located at the center of the nebulizing tip F (i.d. of 0.4 mm). The top of the nozzle, however, is set about 0.3 mm above the nebulizing tip. Through a needle B, nebulizing gas of about 50 mL/min (at atmospheric pressure) was supplied to the nebulizer to form a jet stream at the narrow gap between the nozzle and the tip. The column effluent led to the top of the nozzle is continuously nebulized by the jet stream of He. Depending on experimental conditions such as the volatility of the solutes and boiling point and flow rate of the solvents, the nebulizing tube E (Pyrex) was heated to any desired temperature up to 300 °C, mostly to make up for the latent heat of vaporization of the solvents during nebulization. The distance between the nebulizing tip and the counter orifice (i.d. of 0.5 mm) was adjusted to attain an optimum vapor pressure (ca. 1 Torr) at the CI chamber during full evacuation through G by a rotary pump at a rate of 170 L/min. For example, the optimum distances were about 2.0 and 2.5 mm for 16 μL/min of methanol and water solvent, respectively. In the following, only 16 μL/min of the flow rate was used, but it is possible to use higher

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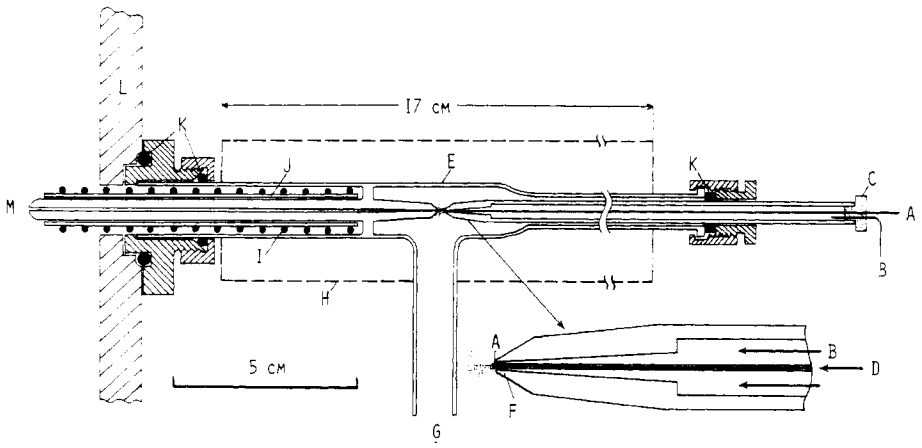

Figure 1. Schematic diagram of vacuum nebulizing interface for LC-MS coupling. (A) coaxial capillary tube from LC, (B) needle for introduction of nebulizing gas (He), (C) silicone septum, (D) effluent from LC, (E) nebulizing tube (Pyrex), (F) nebulizing tip (Pyrex), (G) to rotary pump, (H) heating oven, (I) heater for counter capillary to MS, (J) heater sheath (Pyrex), (K) silicone O-ring, (L) body of MS, (M) to ion source of MS

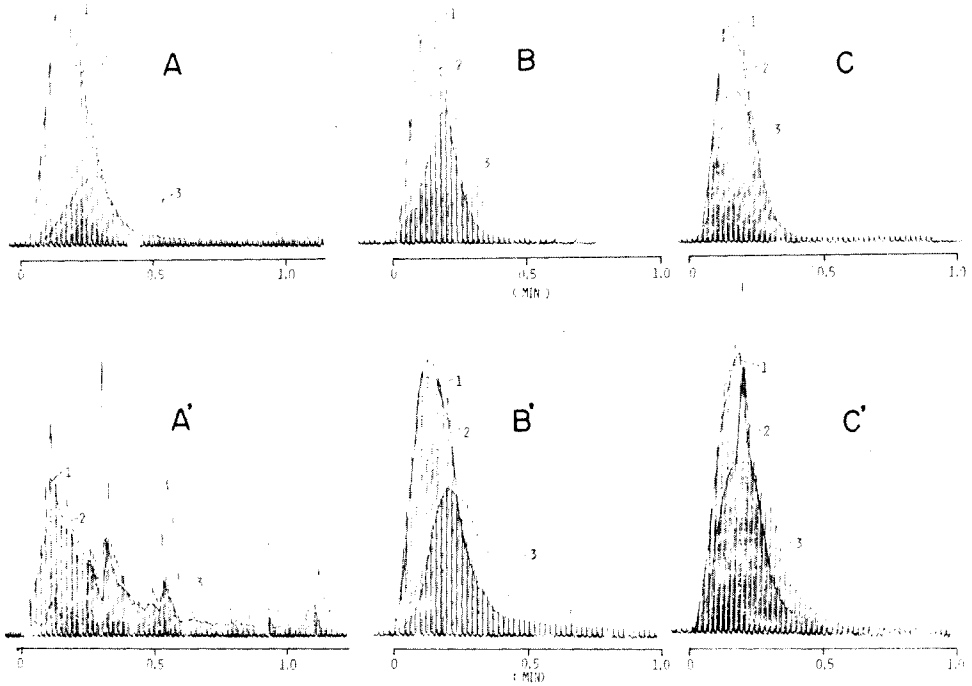

Figure 2. Effect of nebulizing gas and temperature on sample introduction through the vacuum nebulizer. Temperature of the nebulizer: 120 °C for A and A′, 158 °C for B and B′, and 195 °C for C and C′; A, B, and C: using nebulizing gas, and A′, B′, and C′: without using nebulizing gas; sample: 0.1 μL of methanol solution which contains α-naphthylamine (1), diphenylamine (2) and *N*-phenyl-α-naphthylamine (3), 1 nmol of each component

flow rates up to about 100 μL/min by adjusting the evacuation rate, the distance of the nozzle in the nebulizer, and the flow rate of the nebulizing gas. To avoid adsorption of the nebulized small particles of relatively involatile compounds to the inner wall of the countercapillary to the ion source, the capillary was independently heated to any desired temperature up to 300 °C.

Apparatus: A micro LC, FAMILIC-100 from JASCO, with a micro UV-detector, which was developed by Ishii (*10*), was used basically in the same flow system as described previously (*8*), except for the interface. A quadrupole mass spectrometer, JMS-Q10A from JEOLCO was used in the CI mode at 250 eV of the ionizing voltage and 100 μA of the ionizing current.

RESULTS AND DISCUSSION

In our previous work using an ordinary jet separator for GC-MS, the effluent of LC was directly introduced into the separator at 150 °C through a stainless steel capillary (i.d. of 0.1 mm). This separator functioned as a fairly reasonable interface between LC and MS for relatively volatile and stable compounds. With this interface, however, stable and continuous introduction of relatively involatile compounds such as large molecule aromatic amines, cholesterols, polar drugs, and oligopeptides was essentially difficult or almost impossible.

Therefore, in the newly designed interface, the effluent from LC was led to a vacuum nebulizer through a coaxial capillary tube. Here, the core-wire in the capillary plays an important role to make stable the supply of the column effluent to the top of the nozzle with matching the pressure gradient along the capillary; otherwise uniform introduction of the effluent cannot be achieved. Further, this core-wire helps to clean the nozzle after a long run. In the following, about 50 mL/min of He (at atmospheric pressure) was used to make a jet flow at the nebulizing tip.

Figure 2 illustrates the effect of the nebulizing gas on the uniform and stable introduction of the effluent into the ion source. A methanol solution of aromatic amines such as α-naphthylamine (MW = 143), diphenylamine (MW = 169)

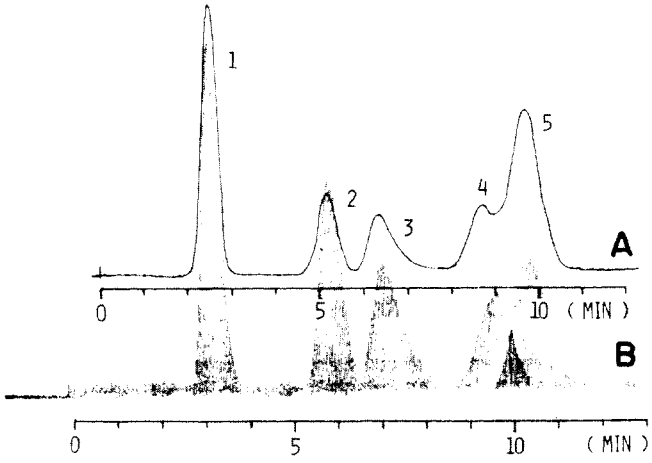

Figure 3. Typical chromatograms of aromatic amines. (A) UV detection at 254 nm, (B) MS detection at $(M + 1)^+$ by repetitive scan within m/e = 140–270, sample: 0.1 μL of methanol solution which contains α-naphthylamine (1), diphenylamine (2), *N*-phenyl-*N'*-isopropyl-*p*-phenylenediamine (3), *N,N'*-diphenyl-*p*-phenylenediamine (4) and *N*-phenyl-α-naphthylamine (5), 1 nmol of each component

and *N*-phenyl-α-naphthylamine (MW = 219) was used as a testing sample. In each case, 0.1 μL of the solution which contains about 1 nmol for each component was injected through the coaxial capillary by a constant flow of methanol (16 μL/min) without passing through any separation column. Mass spectrometric detection was carried out by repetitive scanning (2 s/scan) in a mass range of m/e = 140–230 to cover only the $(M + 1)^+$ peaks. The surface temperature of the nebulizer was maintained at 120 °C for A and A', 158 °C for B and B', and 195 °C for C and C', respectively. The exact temperature of the nebulizing tip, however, is expected to be fairly below the nominal surface temperatures because of the cooling effect owing to the large latent heat of the vaporization of the solvent at the nozzle (for methanol, for example, 8.43 kcal/mol at atmospheric pressure). As can be seen in Figure 2, when nebulizing is used, every solute is introduced in a uniform flow regardless of the temperatures used. At lower temperatures, however, *N*-phenyl-α-naphthylamine whose boiling point is 375 °C/528 Torr shows remarkable time delay, presumably owing to the larger particle size of the nebulized solution at relatively lower temperatures and the higher degree of adsorption to the inner wall of the counter capillary to the ion source. Such time delay for the relatively involatile component becomes almost negligible at the elevated temperature of 195 °C. On the other hand, when the nebulizing gas flow is stopped, the uniform sample introduction is significantly disturbed. This tendency becomes more serious at lower temperatures. Further, the amount of the solute reaching the ion source without the nebulizing gas becomes about one order less than that with the nebulizing gas. Therefore, A', B', and C' in Figure 2 were recorded using 10 times as much gain as in A, B, and C. From the data given in Figure 2, it is inferred that in addition to getting an uniform and stable sample introduction with higher concentration, the use of the nebulizing gas makes possible the use of nominal nebulizing temperatures at least 50 °C lower than those without using the gas. These aromatic amines were introduced in about 80% yield under the following experimental conditions: 50 mL/min of the nebulizing gas, 195 °C of the nebulizing temperature, 2.0 mm of the distance between the nozzles, and 16 μL/min of the methanol solvent.

Figure 3 shows typical chromatograms of aromatic amines such as α-naphthylamine (MW = 143), diphenylamine (MW = 169), *N*-phenyl-α-naphthylamine (MW = 219), *N*-phenyl-*N'*-isopropyl-*p*-phenylenediamine (MW = 226) and

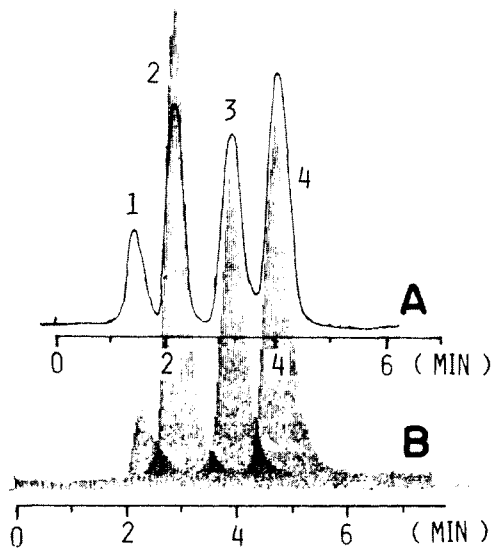

Figure 4. Chromatograms of typical components in a cold medicine. (A) UV detection at 210 nm, (B) MS detection at $(M + 1)^+$ by repetitive scan within m/e = 180–240, sample: 0.1 μL of methanol solution which contains barbital (1), phenacetin (2), aminopyrin (3), and caffeine (4), 1 nmol of each component

N,N'-diphenyl-*p*-phenylenediamine (MW = 260). One-tenth μL of the methanol solution which contains about 1 nmol of each component was separated by a micro-packed column, ODS-SC-01 from JASCO packed in a PTFE tube (i.d. of 0.5 mm × 7 cm length) using 16 μL/min of a mixed solvent (acetonitrile/water, 55/45), and detected successively by the micro UV detector at 254 nm (A) and the MS through the interface (B). The MS detection was carried out by repetitive scanning (2 s/scan) in a mass range of m/e = 140–270 to cover the $(M + 1)^+$ peaks at the nebulizing temperature of 195 °C. About 30 s of the time delay in the mass chromatogram B corresponds to about 8 μL of the additional dead volume between the column and the nebulizer. Under these conditions, however, any additional peak broadening on chromatogram B was not observed. Owing to the discriminating nature of the MS-detection, the overlapped peaks, 4 and 5 on chromatogram A, by UV-detection can be discriminately recorded on mass chromatogram B. This situation is very effective for the detection of poorly resolved or even totally overlapped peaks on the usual chromatograms if they have different molecular weights.

As another application, Figure 4 demonstrates a mass chromatogram of the typical components in a cold medicine; phenacetin (MW = 179), barbital (MW = 184), caffeine (MW = 194), and aminopyrin (MW = 231), together with that obtained by UV-detection at 210 nm. One-tenth μL of the methanol solution which contains about 1 nmol of each component was separated by a micro-packed column, TSK-GEL-111 from Toyo-Soda packed in a PTFE tube (i.d. of 0.5 mm × 23 cm length) using 16 μL/min of methanol. The mass chromatographic detection was carried out by repetitive scanning using 200 °C of the nebulizing temperature.

It is very advantageous that by this detection system almost any kind of usual solvents such as water, hydrocarbons, alcohols, ketones, esters, nitriles, etc., can be used if their vapors act as good reagent gases for CI and give few interfering peaks in the objective mass region of the CI spectrum. If necessary, gradient elution can be adopted. In this case, however, it should be taken into account that the sensitivity for each component might change as a function of the solvent composition.

As mentioned in the introduction, this nebulizing interface can be used as an unique sampling system for polar and/or

large molecules into the CI ion source without using any separation column. In this case, testing solutes of interest are first dissolved in an appropriate solvent which becomes a good reagent gas for CI, and then an aliquot of the solution is introduced into the coaxial capillary by a constant flow of the same solvent from a micro-feeder pump of the LC. With this technique, it was possible to take fairly stable CI mass spectra of polyethyleneglycols up to $m/e = 600$, and many of the free amino acids, oligopeptides, and steroids (9). Further investigation along these lines is under way.

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Double-Reservoir Rotoevaporation Vessel for Residue Analysis

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Advances in chromatographic techniques and instrumentation during the past several years have increased the accuracy, sensitivity, specificity, and efficiency of residue analysis. The automation of gel permeation chromatography (GPC) for lipid-residue separation (1) has significantly increased the number of lipid extracts that can be cleaned up and processed. There has been little improvement, however, in reducing the number of manual transfers involved in processing a sample through extraction and cleanup procedures. Standard techniques for sample preparation include several steps: extraction, lipid removal, and fractionation by sequential adsorption chromatography. Each step involves removal or concentration of large volumes of solvent (2, 3). Solvent removal and quantitative transfer of the residues are time-consuming and limit the number of samples that can be prepared for gas chromatographic analysis within a given period. To use the advantages of rotoevaporation (rapid evaporation under vacuum with little heat) over other methods of organic solvent concentration (e.g., Kuderna-Danish), we designed a double-reservoir rotoevaporation vessel for collection, concentration, and final volume calibration of column eluates (Figure 1). Solvents eluted from our automated GPC system and adsorption chromatography columns are collected directly in the double-reservoir vessels and rotoevaporated to a small volume in the lower reservoir. The vessel walls are then rinsed with a suitable solvent until the volume reaches

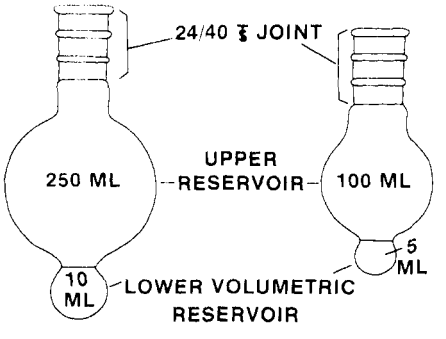

Figure 1. Double-reservoir rotoevaporation vessel

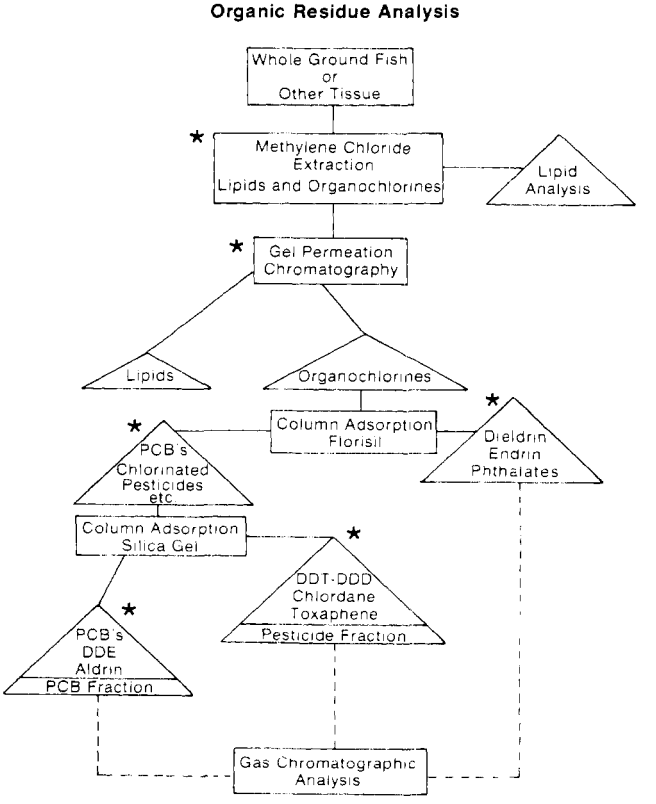

Figure 2. Flow scheme of laboratory procedure. A * designates the steps where a quantitative transfer is eliminated by the use of a double-reservoir rotoevaporation vessel