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MICRO COLUMN LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY USING A CAPILLARY INTERFACE

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SUMMARY

The design and performance of a capillary interface for micro column liquid chromatography–mass spectrometry are described. The total effluent (10–40 μl/min) of the micro column is fed into the ion source of a quadrupole mass spectrometer via a desolvation chamber, whereby the mobile phase provides for chemical ionization. The interface is based on a fused-silica capillary (25 μm I.D.) fitted to a removable probe. The outlet of the capillary is brazed into a bar of brass to achieve an efficient heat transfer from the heated probe tip to the mobile phase. The temperatures of the probe tip, desolvation chamber and ion source are controlled independently. A discharge tube has been installed on the desolvation chamber as an alternative to the filament. Applications to the analysis of sugars, antioxidants and pharmaceuticals are presented.

INTRODUCTION

The combination of liquid chromatography (LC) with mass spectrometry (MS) is becoming increasingly important in industry. However, its realization is not easy, because the operating conditions for the individual techniques are quite different. On the one hand, LC uses pressurized liquids, on the other the mass spectrometer operates under high vacuum.

The vacuum system of a mass spectrometer will accept liquid flows in the range of 10–20 μl/min. For higher flow-rates it is necessary to modify the vacuum system (thermospray interface[1,2]), to remove the solvent before entry into the ion source (moving belt interface[3,4]) or to split the effluent of the column [direct liquid introduction (DLI) interface[5]]. In the latter case only a small fraction (10–20 μl/min) of the total effluent is introduced into the ion source where the mobile phase provides for chemical ionization (CI) of the sample.

The combination of LC with MS is facilitated by using a LC system with microbore columns (1 mm I.D.) instead of conventional columns (4–5 mm I.D.). Flow-rates in LC systems with microbore columns are in the range of 10–50 μl/min, thereby enabling the total effluent to be introduced into the ion source[6–11]. This increases the sensitivity of the method, compared to systems which require splitting of the effluent. In addition, microbore columns are preferred when the sample amount

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is limited or an expensive mobile phase, *e.g.*, deuterated solvents[6], has to be used.

Because of the above advantages of microbore columns, we have used an interface designed for this type of column. In view of the need to handle thermolabile compounds, it should be a DLI rather than a moving-belt interface. Unfortunately, no such interface was commercially available at the time, and so we decided to build our own to the following specifications: removable probe; low dead volume (microbore columns); suitable for daily routine work; capable of handling thermolabile and polar compounds; able to handle solvents used with reversed-phase columns. This report describes the design and performance of the capillary interface and the modification of a desolvation chamber for discharge ionization.

EXPERIMENTAL

Fig. 1 shows a schematic drawing of the micro LC–MS system used in this work. The eluent, delivered by a micro LC pump (a), flows from the injection system (b) through a fused-silica capillary (50 μm I.D.) (c) to the column (d). To minimize the dead volume of the system, the microbore column is partly introduced into the interface (e). Through a fused-silica capillary (25 μm I.D.) (f) with a heated tip, the eluent is fed into the ion source (i) of the quadrupole mass spectrometer via a desolvation chamber (g). The discharge tube[12,13] (h) fitted to the desolvation chamber is an alternative to the filament (k).

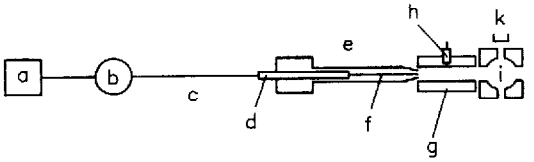

Fig. 1. Schematic diagram of the micro LC–MS system. a = Pump; b = sample injector; c = fused-silica capillary (50 μm I.D.); d = microbore column; e = capillary LC–MS interface; f = fused-silica capillary (25 μm I.D.); g = desolvation chamber; h = discharge tube; i = ion source; k = filament.

LC apparatus

The mobile phase was delivered by a Model 302 pump (Gilson, Villiers-le-Bel, France) set for the flow range of 0.5–500 μl/min. The pressure was monitored by a Gilson Model 802 manometric module, which was used without the pulse-damping unit. Samples were introduced into the system by an Eyela (Tokyo, Japan) sample injector, with six different injection volumes on the same disc, ranging from 0.1 to 1 μl. For the separations a home-made stainless-steel column (25 cm × 1 mm I.D.) was used. It was slurry packed (slurry: tetrachloromethane) with Nucleosil 5 C_{18} (Macherey, Nagel & Co., Düren, F.R.G.). Before the link-up to the mass spectrometer, optimum separation conditions were developed using a Spectroflow 773 UV detector (Kratos, Manchester, U.K.), equipped with a flow cell of 0.5 μl. To test the performance of the LC–MS interface the system was used in the flow-injection analysis (FIA) mode. In this mode the microbore column is replaced by a fused-silica capillary (50 μm I.D.).

Mass spectrometer

The mass spectrometer used was a Nermag R 10-10C quadrupole instrument (Rueil-Malmaison, France), coupled with a Sidar 111B data system. The instrument was equipped with the standard EI–CI source. The pumping capacity at the ion source was reinforced by using a Model 2033 pump (Alcatel, Paris, France) with a flow of 585 l/min instead of 260 l/min. It was thus possible to introduce up to 40 μl/min of pure water or up to 70 μl/min of a mixture of methanol–water or acetonitrile–water into the mass spectrometer.

Chemicals

All solvents used were HPLC grade. Water was purified with a Milli-Q system (Waters Millipore). Samples were obtained from Fluka (Buchs, Switzerland) and Ciba-Geigy (Basle, Switzerland). Prior to use, all solvents and solutions were filtered through an Acrodisc filter (Gelman Sciences, Ann Arbor, MI, U.S.A.) with a pore size of 0.45 μm. The solvents were thoroughly degassed in an ultrasonic bath and permanently flushed with helium.

Capillary LC–MS interface

The capillary interface is shown schematically in Fig. 2. It has the same outside dimensions (1/2 in. O.D.) as the solid probe, and so can be introduced into the mass spectrometer through the solid probe inlet. Basically the interface consists of two stainless-steel tubes. The outer tube (a) is a cover for the partly cut open inner tube (c), which serves as a holder for the microbore column (f), the electric leads and the removable tip. A fused-silica capillary (12 cm × 25 μm I.D.) (n) is fitted with a Valco union (h) to the outlet of the column. Through this capillary the total effluent of the microbore column is introduced into the ion source of the mass spectrometer via a desolvation chamber. To produce a smooth evaporation of the liquid, it is necessary to heat the tip of the capillary. Otherwise the eluent may freeze at the capillary outlet.

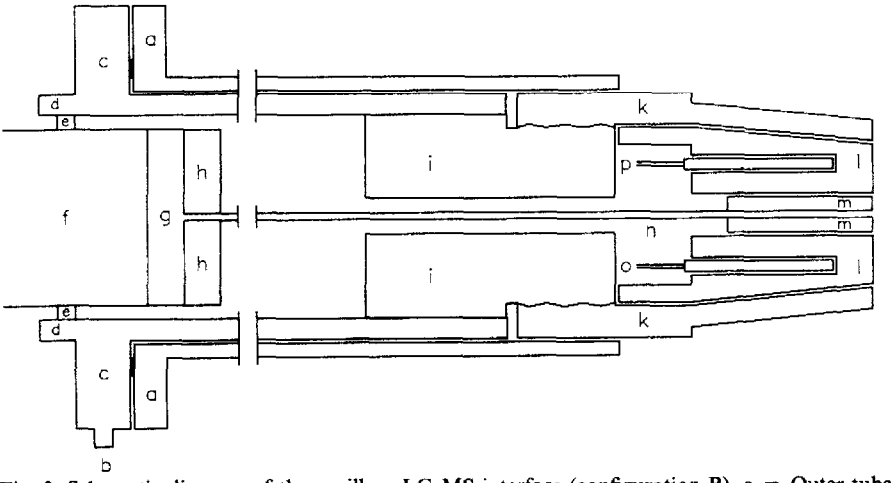

Fig. 2. Schematic diagram of the capillary LC–MS interface (configuration B). a = Outer tube (cover); b = plug for electrical connections; c = inner tube (holder); d = Swagelok fitting; e = ferrule; f = microbore column; g = column end frit; h = Valco union; i = tip holder; k = hull (Vespel); l = copper shells; m = brass bar; n = fused-silica capillary (25 μm I.D.); o = heater; p = temperature sensor.

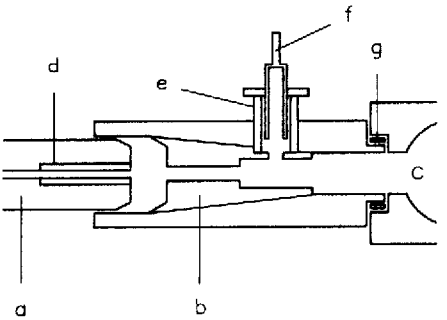

Fig. 3. Schematic view of the desolvation chamber. a = Interface tip; b = step-like insert; c = ion source; d = brazed fused-silica capillary; e, f = discharge tube (e = insulator; f = metallic insert); g = Vespel insulator.

The tip is heated with the aid of two half copper shells (l) fitted to the tip holder (i). The temperature of the tip is measured in one shell by a Pt 100 sensor (p) (Degussa, Switzerland). The heater (o) in the other shell is regulated by a temperature feedback control (Pantatherm D4; Gossen, Erlangen, F.R.G.). The heater consists of a multibore ceramic bar (Degussa) with a heating wire (Bulten-Kanthal, Sweden) wound through the bores. To guarantee good heat transfer from the heated copper shell to the eluent, the capillary is brazed into a round slotted bar of brass (m), with a soft solder (melting point: 301°C; Egli-Fischer, Switzerland). By screwing a Vespel (DuPont) hull (k) to the tip holder, the brass bar and the copper shells are pressed together. This hull also provides thermal and electrical insulation between tip and desolvation chamber.

Desolvation chamber

The desolvation chamber (Fig. 3) was supplied by Nermag. The inner geometry was modified from a conical to a step-like shape (diameter of the steps: 2, 3, 4 mm) by the insertion of a stainless-steel part (b). A discharge tube (e, f) was fitted to the desolvation chamber. The first experiments were carried out with a Finnigan discharge tube, consisting of a ceramic insulator with a metallic insert. This was subsequently replaced by a home-made device. A quartz tube (5 mm O.D., 4 mm I.D.) was used as insulator (e). The metallic insert (f) was made of stainless-steel tube (1/8 in. O.D., 2.5 mm I.D.). A metallic ring was brazed onto the tube to keep it in position. The outer end was then compressed with pliers and the high voltage lead attached. A 2.4-MΩ resistor for current limiting and a multimeter for current measuring were added between the regulated high voltage power supply (Model 507R; Brandenburg, U.K.) and the discharge tube. The desolvation chamber was insulated thermally and electrically from the ion source by a Vespel tube (g).

RESULTS AND DISCUSSION

Our first experiments were accomplished with a simpler version of the capillary interface and without using the desolvation chamber. The tip of the interface was heated indirectly by the ion source. With this configuration the tip had to be in good

contact with the ion source. Consequently it was not possible to adjust the longitudinal position of the probe to optimize the ionization conditions.

An improvement was the installation of a heated desolvation chamber in front of the ion source. The probe tip was then heated by the desolvation chamber. Still we noted that the position of the probe needed to be varied for optimum ionization conditions. We therefore reconstructed the tip of the interface. A probe tip consisting of two copper shells, as well as a heater, temperature sensor and Vespel hull, was built. Heat transfer to the fused-silica capillary was achieved by pressing the two shells firmly against the capillary. However, pressure fluctuations caused by an irregular vaporization of the mobile phase occurred, especially when the water content

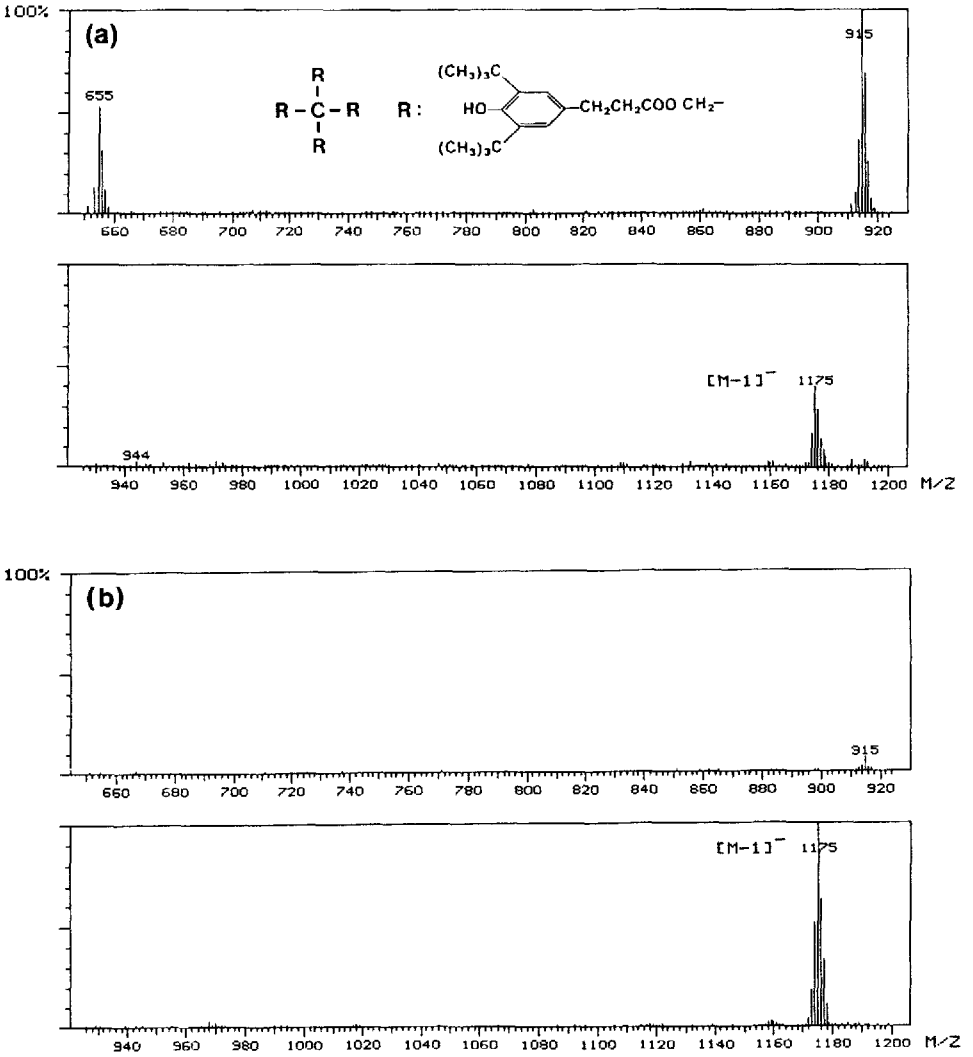

Fig. 4. NCI mass spectra of Irganox 1010: a, obtained with configuration A; b, obtained with configuration B.

was high. The pressure was constant when the end of the capillary was retracted 2–3 mm between the shells (configuration A). With this configuration we often observed fragmentation of the compounds. Another way to avoid pressure fluctuations was to braze the fused-silica capillary into a round, slotted bar of brass which was then pressed between the two shells (configuration B). In this way sufficient heat transfer to the liquid was achieved resulting in a smooth evaporation.

The two mass spectra of the antioxidant Irganox 1010 in Fig. 4 demonstrate the performance of both interface configurations. Spectrum a was obtained with configuration A (bare capillary) and spectrum b by using configuration B (brazed capillary). The former clearly shows two fragment ions with m/z 915 (base peak) and m/z 655 beside the $[M - 1]^-$ ion (m/z 1175). The latter, with base peak $[M - 1]^-$, shows very little fragmentation. This indicates sufficient heat transfer in configuration B without inducing thermal fragmentation.

The length of the brass bar was not very important. We tested different lengths, ranging from 4 to 20 mm without observing any significant difference in the evaporation of the mobile phase or in the fragmentation patterns of different compounds. We are currently using bars of brass with a length of 8 mm.

The temperature of the tip was in most cases between 130 and 220°C, depending on the mobile phase, flow-rate and nature of the compound. For a given sample the temperature range did not appear to be a critical parameter for optimum conditions. The temperatures of the desolvation chamber and ion source were between 220 and 280°C.

To obtain spectra with molecular weight information from a thermolabile compound, such as sucrose, it was necessary to reduce the pressure in the ion source. This was easily done by withdrawing the inlet for the calibration gas, which is located opposite the solid probe port.

Fig. 5 shows the negative CI (NCI) mass spectrum of sucrose with $[M - 1]^-$

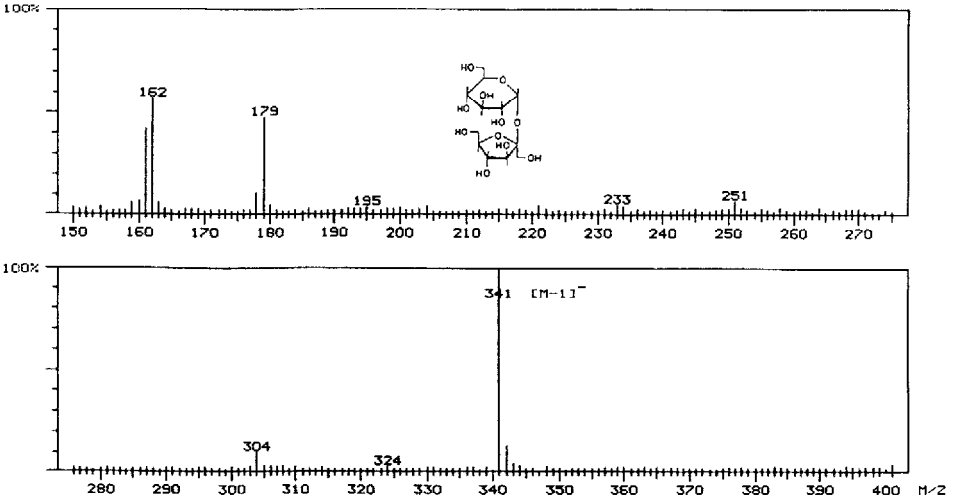

Fig. 5. NCI mass spectrum of sucrose: FIA mode; flow-rate, 25 µl/min; mobile phase, methanol–water (95:5); temperatures, 160, 270, 270°C (probe tip, desolvation chamber, ion source); discharge CI (−2 kV); scan time, 1 sec.

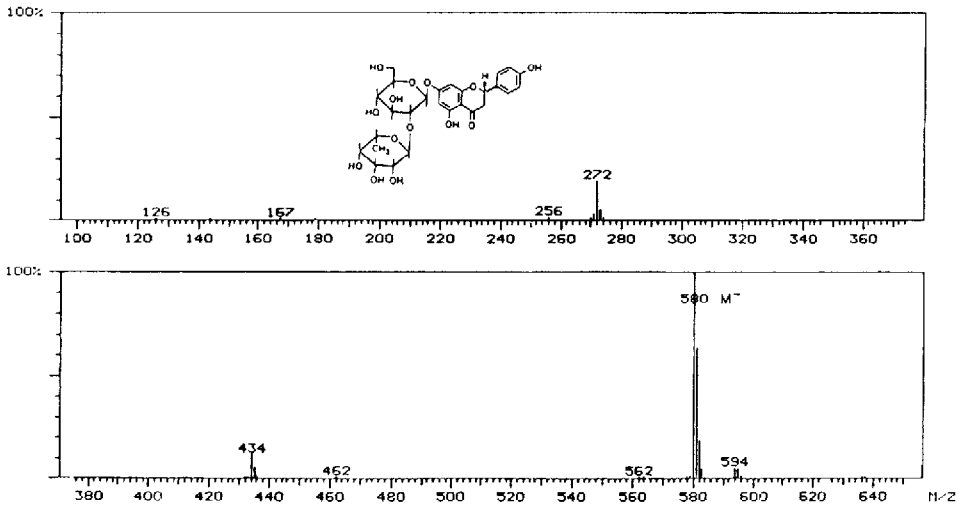

Fig. 6. NCI mass spectrum of naringin. Conditions as in Fig. 5.

as base peak and minimum fragmentation. It is similar to spectra obtained with a DLI interface using a diaphragm as restriction[14]. Likewise the NCI spectrum of naringin (Fig. 6) shows mainly molecular weight information. This demonstrates the absence of thermal degradation.

To eliminate the problem of the short lifespan of the filament due to mobile phases with a high water content or salts, a discharge tube was installed as an alternative. Because we did not want to modify the ion source, the discharge tube was fitted to the upper side of the desolvation chamber. In the event of contamination, the discharge tube can be exchanged very easily, without removing the ion source and desolvation chamber. It was operated in the anodic mode[13], connected to the negative terminal of the high voltage power supply. Best results were obtained when

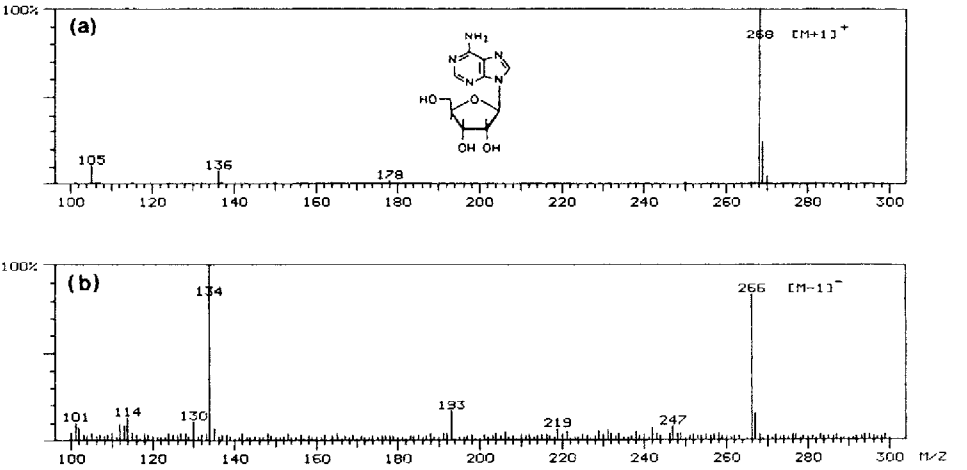

Fig. 7. Mass spectra of adenosine: a, PCI; b, NCI. FIA mode with water as mobile phase, remaining conditions as in Fig. 5.

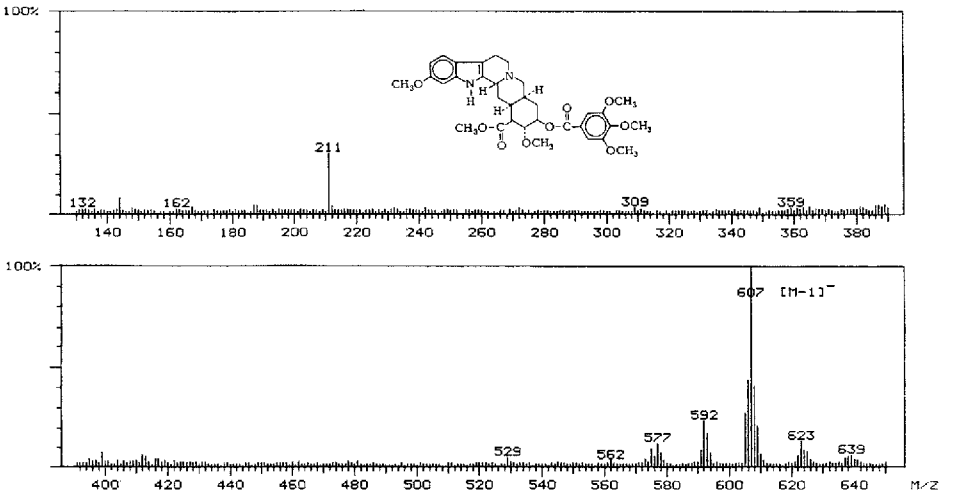

Fig. 8. NCI mass spectrum of Reserpine. Conditions as in Fig. 5.

the desolvation chamber was held at the same potential as the ion source (0–15 V). The discharge generally started in the range of −600 to −1000 V, depending on the flow-rate. The negative voltage was then adjusted for maximum intensity of the background ions (typically −2000 to −2500 V). The corresponding current measured was 300–500 μA. In our configuration the discharge could be used with flow-rates of 12–40 μl/min. Below 12 μl/min it became unstable. To increase the efficiency of the discharge ionization the inner geometry of the desolvation chamber was changed as shown in Fig. 3. The fragmentation pattern observed with the discharge and filament ionization were essentially the same. The sensitivity was higher for the discharge ionization by a factor of 2–5. The discharge ionization allowed the use of a variety of mobile phases, including acids, bases and pure water.

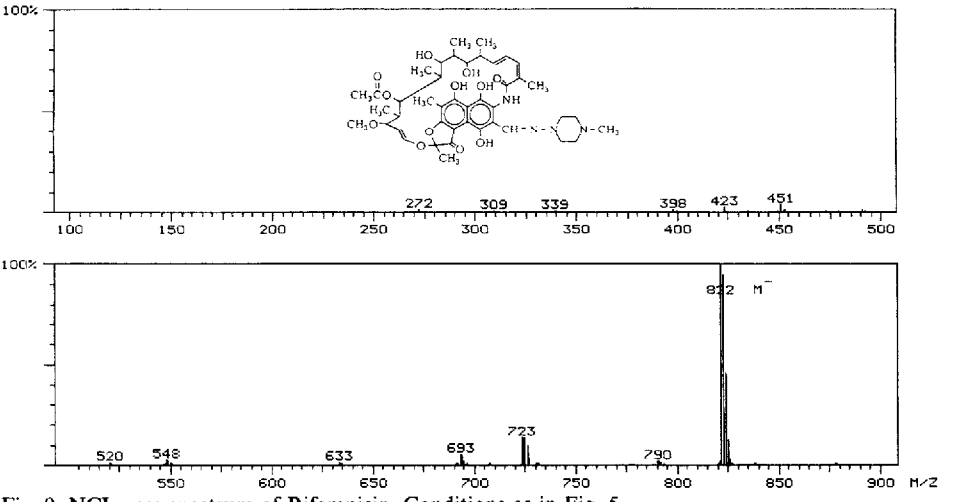

Fig. 9. NCI mass spectrum of Rifampicin. Conditions as in Fig. 5.

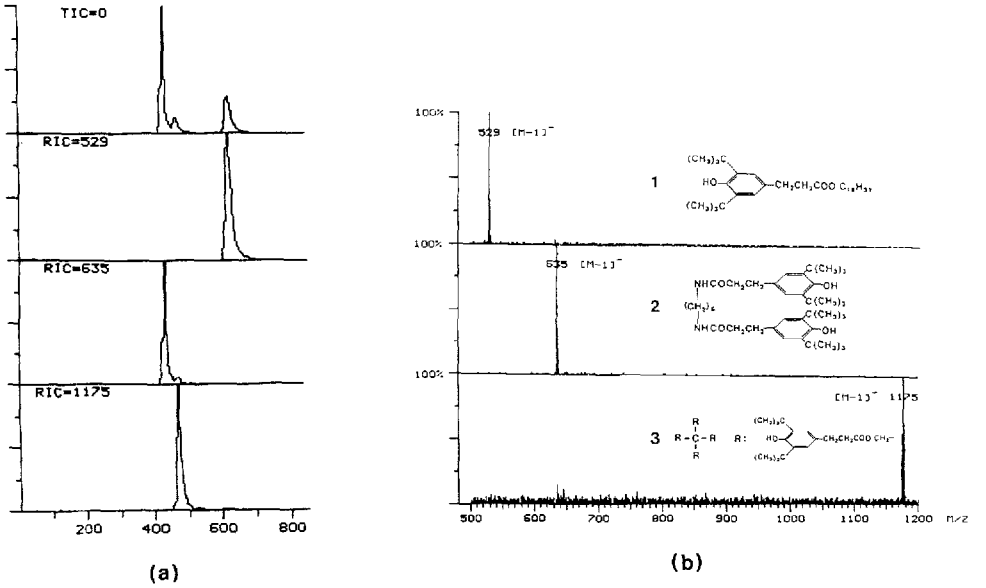

Fig. 10. LC–MS analysis of a synthetic mixture of three antioxidants. Flow-rate: 35 μl/min. Mobile phase: acetonitrile–tetrahydrofuran (50:50). For LC column see Experimental section. 1, Irganox 1076; 2, Irganox 1098; 3, Irganox 1010. a, Total ion current (TIC) and mass chromatograms (RIC); b, mass spectra of the respective chromatographic peaks.

Fig. 7 shows the positive CI (PCI) and NCI mass spectra of adenosine, using water as eluent. Both spectra indicate that there is very little thermal degradation during the evaporation process[2].

To evaluate further the performance of the interface, a variety of samples was investigated (FIA mode) and the spectra were compared to published results. The sensitivity was strongly dependent on the nature of the individual compounds. Generally, for full scan spectra the injection of 10–100 ng was needed. Two examples are presented here. The first is the indole alkaloid tranquillizer Reserpine[2,15], which is known to be difficult to characterize by mass spectrometry. Fig. 8 shows the NCI mass spectrum with $[M - 1]^-$ as base peak and very little fragmentation. PCI spectra of Reserpine were also obtained, however with lower sensitivity, showing m/z 609 as base peak corresponding to $[M + 1]^+$. As a second example the NCI mass spectrum of the antibiotic Rifampicin[14] with m/z 822 (M^-) as base peak is shown in Fig. 9.

Fig. 10 shows the chromatographic separation of a mixture of the three antioxidants Irganox 1098 (MW 636), Irganox 1010 (MW 1176) and Irganox 1076 (MW 530). The mass spectra were obtained under NCI conditions. Fig. 10a shows the computer-reconstructed total ion current (TIC = 0) and the mass chromatograms of the $[M - 1]^-$ ions (RIC 529, 635, 1175). Fig. 10b presents the mass spectra of these antioxidants. Only the range m/z 500–1200 is shown, because all three spectra are very simple, containing only molecular weight information.

CONCLUSIONS

The capillary LC–MS interface described is simple in design. The low dead

volume permits the use of microbore columns. The utilization of a fused-silica capillary as a restriction instead of a diaphragm facilitates the handling of the interface. The generation of a straight and stable jet is much simpler. In spite of the small inner diameter of the capillary, blockage rarely occurs if the solvents and solutes are filtered carefully. However, in the event of a blockage, the capillary can be quickly replaced and in comparison to a diaphragm the replacement is cheap. Discharge ionization eliminates the problem of the short lifespan of the filament due to mobile phases with a high water content or salts. The use of non-volatile buffers will be the subject of further investigations. The overall ionization efficiency is less dependent on the ion-source pressure with the discharge tube compared to the filament.

This capillary interface proved to be applicable to a broad range of samples and mobile phases. In terms of the case of handling thermolabile and polar molecules, it is comparable to the DLI interface using a diaphragm as restriction. For some compounds like Reserpine and adenosine, the spectra, showing less fragmentation, indicate an even better performance.

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