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Microchip for Combining Gas Chromatography or Capillary Liquid Chromatography with Atmospheric Pressure Photoionization-Mass Spectrometry

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We present a microfabricated nebulizer chip for combining atmospheric pressure photoionization-mass spectrometry (APPI-MS) with gas chromatography (GC) or capillary liquid chromatography (capLC). The chip consists of a silicon plate and a glass plate or two glass plates. The chip includes a sample inlet channel, auxiliary gas and dopant inlet, vaporizer channel, nozzle, and platinum heater. The sample eluted from the capLC or GC is mixed with auxiliary gas and dopant (toluene) in the heated vaporizer. The chip forms a confined jet of the sample vapor, which is photoionized as it exits the chip. The analytical performance of GC- and capLC-microchip APPI-MS was evaluated with some polycyclic aromatic hydrocarbons, amphetamines, and steroids. The GC-μAPPI-MS method provides high sensitivity down to 0.8 fmol, repeatability (RSD = 7.5−14%), and linearity (r = 0.9952−0.9987). The capLC-μAPPI-MS method shows high sensitivity down to 1 fmol, good repeatability (RSD = 3.6−8.1%), and linearity (r = 0.9989−0.9992).

One of the current trends in development of analytical systems is toward miniature lab-on-a-chip devices, which can have several advantages such as faster operation, better performance, smaller sample consumption, reduced waste production, and lower cost over those of conventional systems. Most lab-on-a-chip systems are based on microfluidics, combining, for example, pumping, mixing, reactions, sample treatment, separation, and detection. Optical or electrochemical methods, which can be miniaturized, are often used for detection. However, mass spectrometry (MS), due to its high sensitivity and selectivity, has gained increasing interest during the recent years as a detection method used with lab-on-a-chip devices. Although even entire mass spectrometers have been scaled down to centimeter scale,[1] the main interest has been miniaturization of ion sources. Since electrospray ionization (ESI)[2,3] is the ionization method most used in liquid chromatography−mass spectrometry (LC−MS), and it is relatively simple to miniaturize, research on miniaturization has focused almost completely on ESI. Several miniaturized ESI sources produced with various technologies and materials such as glass,[4] silicon,[5] and polymers[6,7] have been presented. Although ESI is usually the method of choice in LC−MS, other ionization methods such as atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI) have their place in the analysis of less polar and nonpolar compounds.

APPI for LC−MS has been increasingly used since its introduction several years ago.[8,9] In APPI, a liquid sample is vaporized by a heated nebulizer and ionized via proton-transfer or charge-exchange reactions using high-energy photons (about 10 eV) for initialization of the ionization process.[10,11] Since commercial APPI and APCI sources are designed to work with flow rates on a scale of 100−1000 μL/min, and miniaturized sources are not available, APPI and APCI have mostly been limited to traditional LC. Nevertheless, in some studies low flow rates have been used with APCI and APPI. APCI has been combined with supercritical fluid chromatography,[12] open tubular liquid chromatography,[13] and capillary electrophoresis (CE).[14] APPI

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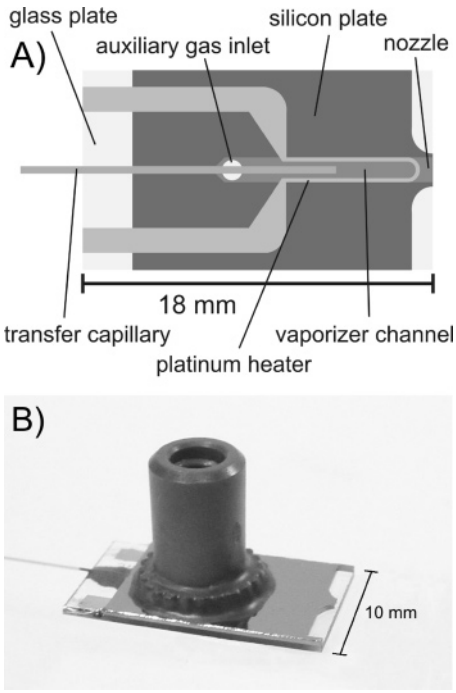

Figure 1. (A) Schematic view of the microchip from the heater side. (B) A photograph of a microchip with Nanoport connector and transfer capillary.

has been used to combine CE[15−17] and capillary electrochromatography (CEC)[18,19] to MS. These sources, however, have been manufactured conventionally or modified from commercial sources and thus lack the advantages of modern microfabrication.

In gas chromatography/mass spectrometry (GC/MS) most of the commercial devices use electron ionization or chemical ionization (CI) under vacuum conditions. APCI was shown to be a potential method for GC/MS over 3 decades ago by Dzidic et al.[20] and was later applied to analysis of various environmental compounds.[21−24] GC-APPI-MS was presented by Revelsky et al. for analysis of a wide variety of analytes including *n*-alkanes, alcohols, ketones, esters, amines,[25] and later for amino acids.[26] Recently McEwen and McKay presented an APCI source modified from a commercial source for use with both LC and GC.[27] The same device functioned as an APPI source by replacing the discharge needle with a UV lamp.[28]

We previously presented a heated nebulizer microchip for APCI and APPI combined with MS.[29−31] We also demonstrated the analytical potential of GC-microchip-APCI[32] (GC-μAPCI-MS) and capillary liquid chromatography (capLC)-μAPCI[33] methods. Here we present a μAPPI-MS method combined to both capLC and GC. We demonstrate the analytical performance of capLC-μAPPI-MS with steroids and that of GC-μAPPI-MS with polycyclic aromatic hydrocarbons (PAHs). The new glass−glass version of the nebulizer chip[34] is used for interfacing liquid chromatography with MS, and its performance is discussed.

EXPERIMENTAL SECTION

Fabrication Process of the Microchips. Here we used two types of nebulizer microchips. The chips used in the GC experiments were of silicon and glass and those used with capLC were of glass only. The silicon−glass nebulizer chips were fabricated with the method presented earlier by our group,[29,31] with minor modifications in design that include removing the capillary stopper structure inside the vaporizer channel and optimizing the position and shape of the heater.

First, a 1 μm thick thermal oxide, which acts as a mask for through-wafer etching, is grown onto a double-sided polished and cleaned silicon wafer. The oxide is etched with buffered hydrofluoric acid (BHF) after double-sided lithography, and the photoresist is stripped. Silicon is etched with deep reactive ion etching (DRIE) first from the channel side and after turning the wafer, from the through-hole side. The heater metallization is patterned on a Pyrex 7740 glass wafer using platinum sputtering with a chromium adhesion layer and lift-off patterning. After removing the mask oxide from the silicon wafer, the wafers are bonded together with anodic bonding. Finally, the wafer stack is diced into individual chips with a wafer saw.

Methyl-deactivated transfer capillaries (SGE, Victoria, Australia) and Nanoport connectors (Upchurch Scientific Inc., Oak Harbor, WA) are glued with high-temperature-resistant epoxy (Duralco 4703; Cotronics Corp., Brooklyn, NY). The capillary is inserted from the rear edge of the silicon plate into the vaporizer channel and glued in place on top of the glass plate. The dimensions of the capillary are 150 μm/220 μm (i.d./o.d.). A schematic drawing of the microchip from the glass wafer side is shown in Figure 1A, and a photograph of a microchip after capillary and Nanoport gluing is shown in Figure 1B.

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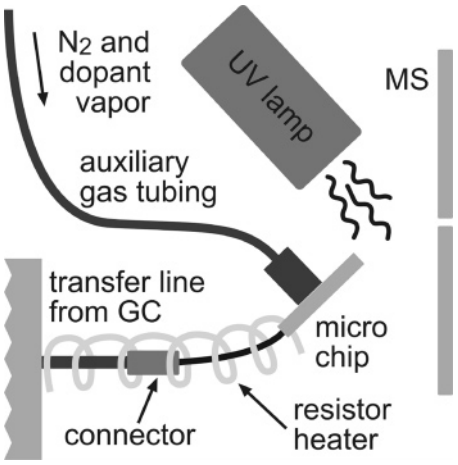

Figure 2. Schematic view of the GC-μAPPI-MS setup.

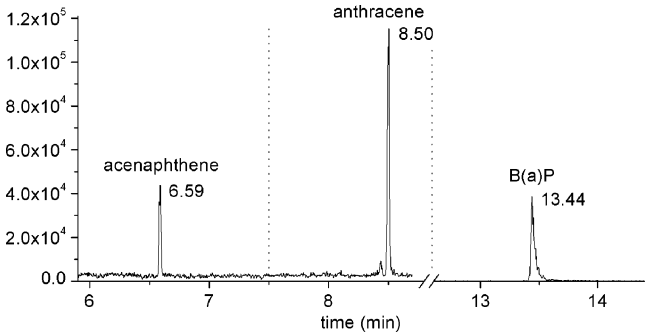

Figure 3. SRM chromatograms of acenaphthene, anthracene, and benzo[*a*]pyrene measured with GC-μAPPI-MS/MS. The injected amounts were 65, 56, and 40 fmol, respectively. SRM pairs were *m*/*z* 154/127 and 154/77 for acenaphthene, *m*/*z* 178/152 and 178/151 for anthracene, and *m*/*z* 252/250 for B[*a*]P.

The glass−glass chip is a new version of the silicon−glass chip. The layout of glass−glass chips is basically the same as that of silicon−glass chips, and all operational parameters (heating power, flow rates, etc.) are similar. Briefly, the manufacturing process of the glass−glass chips is as follows.[34] First a low-pressure chemical vapor deposition (LPCVD) silicon layer is deposited on a Pyrex 7740 wafer. The silicon, which acts as a hard mask in through-wafer glass etching, is patterned with double-sided lithography and isotropic silicon wet etching. After resist stripping, the glass is etched simultaneously from both sides in hydrofluoric acid. The remaining silicon mask is removed in tetramethyl ammonium hydroxide (TMAH), and a blank glass wafer is then fusion-bonded with the etched channel wafer. Platinum heater metallization is patterned using wet etching. In contrast to the silicon−glass chips, the Nanoport connectors are attached after chip dicing with adhesive rings supplied with the Nanoports, instead of high-temperature epoxy. Methyl-deactivated transfer capillaries (SGE) of size 50 μm/220 μm (i.d./o.d.) are used in the glass−glass chips.

Chemicals. The water was purified with a Milli-Q purification system (Millipore, Molsheim, France). High-performance liquid chromatographic (HPLC) grades of methanol, toluene, and chloroform were purchased from Mallinckrodt Baker B.V. (Deventer, The Netherlands), ethyl acetate was from Lab Scan (Dublin, Ireland), acetonitrile was from Rathburn (Rathburn Chemicals, Walkerburn, Scotland), toluene (≥99.9% pure) was from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO), and analytical grade dimethyl sulfoxide (DMSO) was from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany).

The standard compounds testosterone, progesterone, acenaphthene, anthracene, and benzo[*a*]pyrene (B[*a*]P) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Stock solutions of testosterone and progesterone (16 mM) were prepared in a mixture of acetonitrile and DMSO (91/9, v/v), those of anthracene (8.4 mM) and B[*a*]P (5.2 mM) were prepared in ethyl acetate, and that of acenaphthene (7.8 mM) was prepared in methanol. Further dilutions of testosterone and progesterone were done with water/methanol (90/10, v/v), and dilutions of PAHs were done with HPLC grade toluene. Stock solutions (1 mg/mL) of methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), and diphenylamine in methanol were provided by United Laboratories Ltd. (Helsinki, Finland). Further dilutions of these were done with chloroform.

Gas Chromatography. The gas chromatograph was an HP 5890A (Hewlett-Packard, Waldbronn, Germany) equipped with a split/splitless injector and a column transfer line (an MSD transfer line). The GC column was a Varian FactorFour VF5-MS (5% phenyl−95% dimethyl) 15 m × 0.25 mm i.d., 0.25 μm film thickness column (Varian BV, Middelburg, The Netherlands). A 2 m × 0.25 mm i.d. precolumn of methyl-deactivated fused-silica tubing (Varian) was connected with a quartz column connector (Agilent Technologies Inc., Santa Clara, CA). The GC column and the transfer capillary of the microchip were connected with a stainless-steel capillary butt connector and a polyimide ferrule (Sigma-Aldrich, Steinheim, Germany). In addition to the MSD transfer line, we used an additional self-made heated transfer line to heat the end of the GC column and the transfer capillary. A schematic view of the GC-μAPPI-MS setup is presented in Figure 2.

The power source for the additional heater was a programmable dc power supply (ISO-TECH IPS-603A; RS Components, Northants, U.K.), and the temperature of the heater was held at about 310 °C. The temperature was monitored with a K-type thermocouple and a Fluke 54-II thermometer (Fluke Corporation, Everett, MA). The carrier gas was 99.9995% pure helium (Woikoski, Vuohijärvi, Finland) with a 90 kPa column pressure and a flow rate of 1.7 mL/min (at 100 °C oven temperature) corresponding to 58 cm/s. The GC temperature program varied from 65 °C (for 1 min) at 20 °C/min to 320 °C (for 3 min). The samples were injected manually with a 1 min splitless injection. The temperature of the injector and the MSD transfer line was 330 °C.

Liquid Chromatography. The liquid chromatograph was an 1100 series Capillary Liquid Chromatography system (Agilent Technologies, Waldbronn, Germany) with a SymmetryShield RP18 column (100 mm × 0.3 mm i.d., particle size 3.5 μm) from Waters (Milford, MA). The mobile phase consisted of water (A) and acetonitrile/water (90/10, v/v) (B) with the following gradient elution optimized for rapid separation: 15% → 65% B from 0 to 1 min, 65% → 95% B from 1 to 7 min, 95% → 100% B from 7 to 8 min, and 100% → 15% B from 8 to 8.5 min, followed by a 20 min equilibration period at 15% B. The flow rate of the eluent was 9 μL/min, and the column oven was maintained at 50 °C. Samples (1 or 3 μL) were injected by an autosampler with an 8 μL loop.

Table 1. Limits of Detection, Linear Ranges, Linearities, Fitting Parameters, and Repeatabilities of the Compounds Studied with GC-μAPPI-MS

	LOD (S/N = 3)				
	nM	fmol	linear range (nM) and linearity (r)	slope (cps/nM), intercept (cps), and no. of points	repeatability (RSD, $N = 7$)
acenaphthene	13	13	32−3200, 0.9963	797, 16 600, 7	7.5% (130 nM)
anthracene	2.8	2.8	2.8−280, 0.9987	2600, 16 200, 7	14% (110 nM)
benzo[a]pyrene	0.79	0.79	0.79−4000, 0.9952	384, 52.7, 12	11% (79 nM)

The transfer capillary of the microchip was connected directly to the LC column with a NanoTight fitting (Upchurch). The schematic view of the LC-μAPPI-MS measurement setup is the same as that of the GC-μAPPI-MS (Figure 2), except the resistor heater and the transfer line were not used.

Mass Spectrometry. The mass spectrometer was a PE Sciex API 3000 triple-quadrupole (MDS Sciex, Concord, Canada). A Whatman 75-72 nitrogen generator (Whatman Inc., Haverhill, MA) produced the purified nitrogen used as curtain and auxiliary gas. We positioned the microchip in front of the mass spectrometer about 1 cm from the curtain plate with the aid of a manual *xyz* positioning stage. A krypton dc discharge UV lamp with 10 eV photon energy (PKS 100; Cathodeon, Cambridge, England) used for photoionization was situated perpendicular to the microchip. The lamp was installed in a Vespel holder and powered with a custom-made APPI power source (Electronics Facility and Mechanical Shop, University of Groningen, The Netherlands). The power source connects the high-voltage output of the MS, which normally gives the APCI or ion spray voltage, to a repeller electrode in front of the UV lamp in the lamp holder. The repeller voltage was optimized at 1300 V.

A mass flow controller (model GCF17; Aalborg, Orangeburg, NY) controlled the auxiliary gas flow into the microchip. The dopant (≥99.9% pure toluene) was fed coaxially through a capillary into the auxiliary gas tubing with a syringe pump (PHD 2000; Harvard Apparatus, Hollston, MA). The microchip was heated with a dc power supply (EPS EP-6515; Sankyo Kogyo Corp., Japan). In measurements done with the GC, we used 2.5 W chip-heating power, 110 mL/min auxiliary gas flow rate, and 5 μL/min dopant flow. In the capLC experiments, the parameters were 4.0 W, 100 mL/min, and 4 μL/min, respectively. The data were acquired with Analyst 1.4 software.

RESULTS AND DISCUSSION

The auxiliary gas (N_2) flow rate, dopant (toluene) flow rate, and heating power of the chip significantly affected the signal intensity and stability and were therefore optimized for the GC-μAPPI-MS and capLC-μAPPI-MS measurements. The effect of flow rates of dopant (0−10 μL/min) and auxiliary gas (30−300 mL/min) on the sensitivity was studied using anthracene as a test compound in GC-μAPPI-MS experiments and testosterone and progesterone in capLC-μAPPI-MS optimization. The GC carrier gas and capLC eluent flow rates were 1.7 mL/min and 9 μL/min, respectively. The maximal signal intensity in both GC-μAPPI-MS and capLC-μAPPI-MS was achieved with an auxiliary gas flow rate of about 90−120 mL/min, while lower or higher flow rates caused significant decreases in signal intensity. The use of dopant was necessary in both GC-μAPPI-MS and capLC-μAPPI-MS experiments, since the signal intensity was substantially lower without dopant. The flow rate of the dopant also had a significant effect on sensitivity. Increasing the dopant flow rate from 1 μL/min increased the signal until it reached a plateau at about 4 μL/min, above which no increase in signal intensity was detected. Robb and Blades previously observed the same type of activity in a commercial APPI source.[35]

The heating power in the GC experiments was set at 2.5 W, corresponding to a temperature of about 290 °C in the vaporizer channel. This temperature was high enough to ensure elution of the nonvolatile B[a]P (bp 496 °C) in the GC-μAPPI-MS experiments. The epoxy glue used to attach the Nanoport connector to the silicon−glass chip limits the practical heating power below 3 W in long-term operation, since the Nanoport may detach from the chip at higher heating power. Detachment of the Nanoport is not a problem with the new glass−glass chip used in LC-μAPPI-MS, and thus heating powers up to 5 W can be used. This is because the thermal conductivity of the glass−glass chip is significantly lower than that of the silicon−glass chip and the Nanoport area remains at a lower temperature than in silicon−glass chips.[34] This is crucial, since the heating power in capLC-μAPPI-MS substantially affected signal stability, and a stable vaporization process was achieved only at heating powers higher than 3 W. Below 3 W, the vaporization process was unstable, which was recognized as unstable LC−MS ion chromatograms. Since heating powers that are too high may increase thermal dissociation of analytes, the optimal heating power in the LC-μAPPI-MS experiments was 4 W, corresponding to about 370 °C in the vaporizer channel.

GC-μAPPI-MS. The mass spectra of the PAH standards acenaphthene, anthracene, and B[a]P showed intense radical cations and negligible fragmentation, indicating that the ionization mechanism was charge exchange through the following reactions presented earlier:[10]

$$C_7H_8 \text{ (dopant)} + 10 \text{ eV photons} \rightarrow C_7H_8^{\bullet+} + e^-$$

$$C_7H_8^{\bullet+} + M \text{ (analyte)} \rightarrow M^{\bullet+} + C_7H_8$$

The most intense product ions of the radical cations were chosen for selected reaction monitoring (SRM) in the GC-μAPPI-

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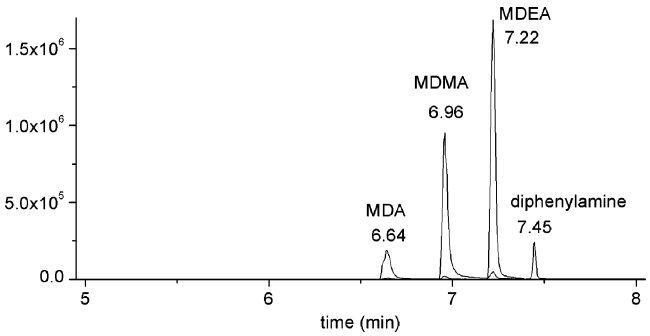

Figure 4. SRM chromatograms of MDA, MDMA, MDEA, and diphenylamine GC-μAPPI-MS/MS. The amounts injected were 51, 48, 44, and 0.59 pmol, respectively. SRM pairs were *m*/*z* 180/163 for MDA, *m*/*z* 194/163 for MDMA, 208/163 *m*/*z* for MDEA, and *m*/*z* 170/93 for diphenylamine.

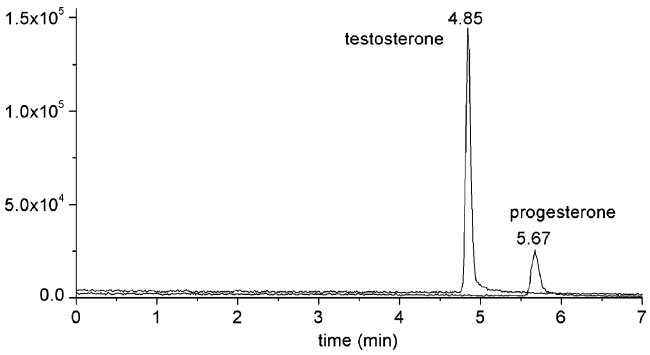

Figure 5. SRM chromatograms of testosterone and progesterone measured with LC-μAPPI-MS/MS. The amount injected was 15 fmol of both compounds. SRM pairs were *m*/*z* 289/109 and 289/97 for testosterone and *m*/*z* 315/109 for progesterone.

tandem mass spectrometry (MS/MS) experiments. For acenaphthene the selected precursor/fragment pairs were *m*/*z* 154/127 and 154/77, for anthracene *m*/*z* 178/152 and 178/151, and for B[*a*]P *m*/*z* 252/250. Figure 3 shows the SRM chromatograms of the PAH standards measured by the GC-μAPPI-MS/MS setup. The injection volume was 1 μL, and concentrations of acenaphthene, anthracene, and B[*a*]P were 65, 56, and 40 nM, respectively.

Figure 3 shows good chromatographic performance with very narrow peaks of acenaphthene and anthracene, both of which have a peak width at half-height of 1.0−1.1 s, which is less than or equal to the typical peak width of PAHs obtained with conventional GC/MS methods.[36] The main reason for the narrow peaks is that the internal volume of the vaporizer channel inside the chip is only about 2 mm^3, which is flushed in less than 2 ms by the auxiliary gas. The peaks of acenaphthene and anthracene are also symmetric, the asymmetry factors (A_s) being 0.98 and 0.89, indicating that no significant adsorption onto the vaporization channel of the chip occurs. However, the peak of B[*a*]P tails (A_s = 2.95) and is broader than the first two peaks, which can be recognized in conventional GC/MS analysis, as well.[37,38] Even then, adsorption onto the microchip cannot be fully excluded since the temperature inside the chip is slightly below the elution temperature of B[*a*]P, which is 310 °C.

The quantitative performance of the GC-μAPPI-MS/MS system using SRM was evaluated by determining the limit of detection (LOD), linearity, and repeatabilitiy for the PAH standards (Table 1). The LODs were determined by measuring a series of samples of decreasing concentrations. The concentration giving a signal-to-noise ratio (S/N) of at least 3 was chosen as the LOD for each compound. LODs were from 0.79 nM of B[*a*]P to 13 nM of acenaphthene (from 0.79 to 13 fmol injected) and were lower than or equal to those reported with conventional GC/MS/MS methods using electron ionization.[39−41] In comparison to our previous GC-μAPCI-MS method,[32] the new μAPPI method is over 2 orders of magnitude more sensitive. The difference is partly due to the higher sensitivity of the mass spectrometer used in this study. Although the analytes are not the same, the new GC-μAPPI-MS method shows better performance than the GC-μAPCI-MS method.

The correlation coefficients (*r*) of the calibration curves within the concentration range examined (0.79−4000 nM) were between 0.9952 and 0.9987, indicating favorable linearity of the method (Table 1). The linear range of B[*a*]P covered almost 4 orders of magnitude, and that of anthracene and acenapthene was 2 orders of magnitude. The repeatability of the method was measured at concentrations of 130, 110, and 79 nM acenapthene, anthracene, and B[*a*]P, respectively. The relative standard deviations (N = 7) were below 15%, indicating acceptable repeatability. Note that the injections were performed manually without the use of an internal standard, and thus a significant proportion of the repeatability was probably due to variation in the injections.

The feasibility of GC-μAPPI-MS/MS for use in qualitative analysis of polar compounds was tested with a set of amphetamine derivatives (MDA, MDMA, and MDEA) and diphenylamine. In the measurements a mixture of dopant (toluene) and methanol (50/50, v/v) was added to the auxiliary gas at a flow rate of 8 μL/min. The spectra of the amphetamine derivatives showed significant fragmentation, but abundant protonated molecules also formed throughout the following reactions:[10]

$$C_7H_8 + 10\ \text{eV photons} \rightarrow C_7H_8^{\bullet +} + e^-$$

$$C_7H_8^{\bullet +} + (CH_3OH)_n \rightarrow C_7H_7 + [(CH_3OH)_n + H]^+$$

$$[(CH_3OH)_n + H]^+ + M\ (\text{analyte}) \rightarrow MH^+ + (CH_3OH)_n$$

The intensity of the protonated molecules increased, and the degree of fragmentation decreased, using the toluene/methanol mixture rather than toluene only. When only toluene is used, the proton-transfer reaction occurs directly between a toluene radical cation and the analyte. The addition of methanol alters the reagent ion composition, and instead of toluene radical cations, the protonated methanol clusters act as proton donors. Since the proton affinity of methanol clusters is higher than that of a toluene radical cation (831 kJ/mol),[9] the exothermicity of the proton-transfer reaction is higher, and therefore, the fragmentation

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Table 2. Limits of Detection, Linear Ranges, Linearities, Fitting Parameters, and Repeatabilities of the Compounds Studied with capLC-μAPPI-MS

	LOD (S/N = 3)		linear range (nM) and linearity (r)	slope (cps/nM), intercept (cps), and no. of points	repeatability (RSD, 10 nM, 1 μL, N = 6) (%)	repeatability (RSD, 50 nM, 3 μL, N = 6) (%)
	nM	fmol				
testosterone	1	1	1−500, 0.9989	27 900, 16 000, 6	11	3.6
progesterone	2	2	5−1000, 0.9992	9190, −3940, 6	16	8.1

is stronger without than with methanol. In general, the degree of fragmentation and selectivity in GC-μAPPI-MS can be controlled by adding suitable volatile solvents or buffers into the auxiliary gas in the same way as by choosing a reagent gas in conventional CI.

Figure 4 shows SRM chromatograms resulting from GC-μAPPI-MS/MS analysis of MDA, MDMA, MDEA, and diphenylamine. The concentrations were 51, 48, 44, and 0.59 μM, respectively. Amphetamines are usually derivatized prior to GC analysis but can also be analyzed without derivatization, which is the case here. However, due to the basic nature of amphetamines, their interactions with the stationary phase are relatively strong, which leads to peak broadening and tailing.

capLC-μAPPI-MS. The performance of capLC-μAPPI-MS/MS was tested with two steroids: testosterone and progesterone. The spectra showed intense protonated molecules and minor fragment ions. The ionization process is similar to that presented above. The proton-transfer reaction occurs via protonated eluent cluster molecules, which were observed as the background ions instead of the toluene radical cations. Figure 5 shows chromatograms measured in the SRM mode using a 5 nM sample and a 3 μL injection volume. The monitored ion pairs were m/z 289/109 and 289/97 for testosterone and m/z 315/109 for progesterone.

The chromatograms show good stability of the method, resulting from use of the glass−glass chip, which can be heated as high as 500 °C, providing efficient and stable vaporization of the eluent and the analytes. The peak widths at half-height of the testosterone and progesterone peaks in Figure 5 are 4.2 and 7.1 s, respectively, which are typical for capLC separations.[42] The asymmetry factors are acceptable: 1.39 for testosterone and 1.61 for progesterone. However, the chromatograms show some peak tailing, which is most likely due to dead volume in the transfer capillary connection to the column. The microchip itself should cause no tailing, due to the high temperature and small dead volume of the vaporizer channel of the chip, which is flushed by the auxiliary gas in less than 2 ms. A significant improvement in peak shape compared to that of the previous capLC-μAPCI-MS work is seen, albeit the present microchip was used without deactivation.[33] This is most likely due to increased chip temperature allowed by the new glass−glass chip, better positioning of the transfer capillary inside the chip, and minimized dead volumes in the column−capillary connection.

Table 2 shows the quantitative performance of the LC-μAPPI-MS/MS system in analysis of testosterone and progesterone using a 1 μL injection volume. The criterion for LODs was the same as in GC measurements. The LODs were 1 and 2 nM (1 and 2 fmol injected) for testosterone and progesterone, respectively, which are equal to or lower than the LODs of testosterone analyzed by conventional LC-APPI-MS/MS[43] and LC-ESI-MS/MS[44] methods. With our previous capLC-μAPCI-MS method, the LOD of testosterone was 10 nM (20 fmol injected).[33] Taking into account the sensitivity difference of the mass spectrometers used in these studies, the new capLC-μAPPI-MS method is more or equally sensitive.

The linearity of the method measured with concentrations between 1 and 1000 nM is good, as shown by the correlation coefficients (r) of 0.9989 and 0.9992. The linear ranges covered over 2 orders of magnitude. The repeatabilites are below 10% at 50 nM concentration and below 20% at a concentration near the limits of quantification (about 5 nM). Overall, the results show good quantitative performance.

CONCLUSIONS

We have demonstrated the suitability of the heated nebulizer microchip for combining GC or capLC with APPI-MS. The results show that the GC-μAPPI-MS and capLC-μAPPI-MS methods are suited well for qualitative and quantitative analysis of nonpolar and polar compounds. As shown previously,[32,33] the chip can be used without modifications in GC- and capLC-APCI-MS, while the change from APPI to APCI involves only replacing the UV lamp with a corona discharge needle. Furthermore, the microchips can be used with any mass spectrometer equipped with an atmospheric pressure ion source. The chips widen the opportunities to combine high-voltage separation systems such as CE or CEC with APPI- or APCI-MS, which previously has been achieved with conventional ion sources.[15,16,18] Since we have shown that both μAPCI and μAPPI perform well with GC and capLC, it may soon be possible to construct a microfabricated heated nebulizer device with an integrated liquid- or gas-phase separation system.

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