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Sonic Spray Ionization Technology: Performance Study and Application to a LC/MS Analysis on a Monolithic Silica Column for Heroin Impurity Profiling

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Sonic spray (SS) ionization is a relatively novel atmospheric pressure ionization technique for LC/MS, based on the principle of "spray ionization", which only recently became commercially available. In this paper, we evaluate the performance of this ion source as an interface for LC/MS in comparison with the more traditional and better studied pneumatically assisted electrospray or ion spray (IS). The effect of organic modifiers, volatile acids, and buffer systems in the LC eluent on the ionization efficiency of both interfaces is described and some possible explanations for the observed phenomena are highlighted. We could conclude that the presence of organic solvents gradually increased the ionization efficiency for IS and SS, while volatile acids or buffers gave a significant signal suppression. Furthermore, we present the application of the sonic spray interface to a fast LC/MS analysis, for the simultaneous determination of the seven prime opium alkaloids in heroin impurity profiling. Chromatographic separation is performed in 5 min on a monolithic silica column (Chromolith Performance) with a gradient elution system and an optimized flow of 5 mL/min. By means of a postcolumn split of ∼1/20, a coupling between the fast LC system and the mass spectrometer is made. The method is validated and successfully applied to the analysis of real-time seized heroin street samples.

In recent years, liquid chromatography/mass spectrometry has become more and more popular, and also in opiate analysis.[1] Indeed, high-performance liquid chromatography is the technique that can separate a wide range of analytes without any chemical pretreatment,[2] while mass spectrometry, used as a detector for the latter separation technique, offers universality and selectivity.[3] Coupling both techniques by specially developed LC/MS interfaces helped to overcome the previous problem of poor sensitivity. In particular, thermospray (TS),[4] electrospray (ES),[5] pneumatically assisted electrospray or ion spray (IS),[6] and atmospheric pressure chemical ionization (APCI)[7] interfaces have been successfully applied to the measurement of opiates and their metabolites.

Sonic spray (SS) ionization, however, is a relatively novel atmospheric pressure ionization (API) technique, developed in the beginning of the 1990s, which only recently became commercially available. In this technique, the column effluent is sprayed from a fused-silica capillary with a sonic velocity gas flow coaxial to the capillary.[8,9] After nebulization of the column eluent, charged droplets and finally gas-phase ions are formed under atmospheric pressure. The ion intensity strongly depends on the gas velocity and reaches a maximum at the sonic velocity (gas velocity ∼1 Mach number), ergo sonic spray ionization. Subsequently, the ions are introduced through a sampling orifice into a vacuum region where mass analysis can be performed. SS is a "soft" ionization interface for liquid-phase separators such as LC and mass spectrometry, which can be used for a broad range of compounds with a high reproducibility. Moreover, since it is not necessary to apply much heat or an electrical field to the capillary of the ion source, sonic spray can also be applied to the analysis of (thermally) labile compounds. So far it has been successfully applied only to a limited number of compounds in the environmental and bioanalytical field.[8−12]

Furthermore, mass analyzers such as quadrupole (Q),[13] triple quadrupole (QQQ),[14,15] and time of flight (TOF)[16] have proven to

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be applicable in opiate analysis. Up until now, a quadrupole ion trap mass spectrometer coupled to HPLC has not been applied in opiate analysis. However, due to the ability to store ions and manipulate ions in time rather than space, ion trap-based mass spectrometers offer unique advantages over more traditional mass spectrometers in which ions pass through the instrument as a beam.[17,18] The biggest advantage is the capability to perform multistage MS (MS^n), which is a powerful tool for structure elucidation and essential for selectivity in the analysis of complex biological matrixes. Furthermore, ion trap mass spectrometers show a full-scan sensitivity, clearly better than those of single quadrupoles. Finally, they are easy to use and to maintain.

Nowadays, laboratories are confronted with the continuously increasing demand for higher sample throughput, thus shorter analysis time. To this aim, monolithic silica HPLC columns are getting a lot of attention. Manufacturing of this new type of columns is performed according to a sol−gel process.[19] This column preparation technique is based on the hydrolysis and polycondensation of alkoxysilanes in the presence of water-soluble polymers. The result is a so-called "rod" made of a single piece of porous silica with a defined bimodal pore structure, i.e., macro- and mesopores. On one hand, the macropores (2 μm in diameter) act like large through-pores or channels, leading to higher permeability, thus low flow resistance. On the other hand, the mesopores (13 nm in diameter), present on the exterior of the monolithic skeleton, provide a large surface area for chromatographic adsorption and desorption processes. As a result, these columns are characterized by high separation efficiency and low column back pressure, making it possible to operate the monolithic silica columns at high flow rates but still maintain an excellent chromatographic performance.[20,21]

In this paper, we evaluate the overall performance of sonic spray ionization as an interface for LC/MS in comparison with the more traditional and better studied pneumatically assisted electrospray or ion spray. The influence of the organic modifiers methanol and acetonitrile, formic acid as a volatile acid, and buffer systems such as ammonium formate and acetate in the LC eluent on the ionization efficiency of both ion sources is investigated. Although the ionization mechanism is influenced by a large number of parameters, an effort is made to highlight some of the major contributors of the observed effects. Furthermore we present the development of a fast LC/SSI-ion trap MS method for the simultaneous determination of the seven prime opium alkaloids present in illicit heroin street samples, i.e., acetylcodeine, 6-monoacetylmorphine (6-MAM), codeine, heroin, morphine, noscapine, and papaverine. A high-speed reversed-phase HPLC method on a monolithic derivatized silica column (Chromolith Performance, 100 × 4.6 mm) is coupled to the optimized MS system by means of a postcolumn split. The method is fully validated by determination of the following parameters: limit of detection (LOD) and quantitation (LOQ), linearity range, within- and between-day reproducibility, accuracy, and selectivity. Regression analysis, using at least six calibrator samples for each compound, ranging from 5 to 4000 ng/mL is presented. Finally, the applicability of the method is demonstrated by the analysis of authentic seized heroin street samples for heroin impurity profiling purposes.

EXPERIMENTAL SECTION

Apparatus. Chromatography was carried out using a LaChrom separation module (Merck, Darmstadt, Germany) including a L-7100 low-pressure gradient pump, L-7200 autosampler (injection loop 100 μL), L-7360 column oven, and D-7000 interface. The system uses the LC/3DQ-MS System Manager Software running under Windows NT version 4.0 on a Compaq Deskpro EN.

All MS experiments were carried out on the M-8000 ion trap-based mass spectrometer from Merck equipped with a pneumatically assisted electrospray or ion spray and a sonic spray interface, both on-axis with the sampling orifice of the mass spectrometer, and operated in positive ion mode. All experiments were performed using the "automatic sensitivity control" (ASC) facility to automatically adjust the accumulation time as the ion abundance changed. Instrument control, data acquisition, and data control were performed using the same software as for the HPLC. The direct transfer from the obtained data to Excell is performed by "dynamic data exchange" (DDE), a special function incorporated in the LC/3DQ-MS System Manager Software.

Reagents. The opiates studied were acetylcodeine, codeine, heroin, 6-monoacetylmorphine, morphine, noscapine, and papaverine, all of which were supplied by Sigma-Aldrich Chemicals (Bornem, Belgium). The internal standard, levallorphan, was a generous gift from Professor R. B. Taylor from The Robert Gordon University (Faculty of Health and Food, Aberdeen, United Kingdom). Ammonium acetate, formic acid, and acetic acid were purchased from Merck-Eurolab (Leuven, Belgium). Ammonium formate was obtained from Sigma-Aldrich Chemicals. Water, methanol, and acetonitrile were all of HPLC gradient grade (Merck-Eurolab).

Sample Preparation. An individual standard solution of 1 g/L of each opiate was prepared in methanol or acetonitrile, according to the solubility of the solute, and stored in the dark at −20 °C until use. Under these conditions, all solutions proved stable for more than 6 months. Working solutions containing a mixture of the opium alkaloids in concentrations ranging from 2 ng to 40 μg/mL (50 μL injected on-column (oc)) were prepared by appropriate dilution with a mixture of water−acetonitrile (50:50, v/v). For regression analysis, each sample (100 μL) was additionally spiked with the internal standard, levallorphan (10 μL of a 20 μg/mL solution, 90.9 ng oc).

Stock solutions of the illicit heroin street samples were prepared by dissolving ∼10 mg of the sample in 10 mL of a mixture of methanol−acetonitrile (50:50, v/v). Subsequently, each sample was appropriately diluted with the mixture water−

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Table 1. Precursor Ion Information and Validation Data of the Opium Alkaloids and the Internal Standard

compd	precursor ion $[M + H]^+$	LOD (ng oc)[a]	LOD (ng/mL)	LOQ (ng oc)[a]	LOQ (ng/mL)	linear range (ng/mL)	regression analysis[b] ($n = 8$)	within-day[c] (CV%)	between-day[d] (CV%)	accuracy[e] (%)
morphine	286	0.5	10	1	20	20−4000	$2.197 \times 10^{-4}x + 1.430 \times 10^{-2}$ ($R^2 = 0.996$)	9.5	20.3	91.6/115.5
codeine	300	0.5	10	1	20	20−4000	$2.861 \times 10^{-4}x + 2.897 \times 10^{-2}$ ($R^2 = 0.990$)	16.8	17.0	85.4/101.0
6-MAM	328	0.5	10	1	20	20−4000	$2.863 \times 10^{-4}x + 9.701 \times 10^{-3}$ ($R^2 = 0.998$)	11.2	17.4	88.4/95.2
papaverine	340	0.1	2	0.25	5	5−1000	$7.825 \times 10^{-4}x + 4.798 \times 10^{-2}$ ($R^2 = 0.992$)	7.9	12.3	108.9/80.2
heroin	370	1.0	20	2	40	40−4000	$2.773 \times 10^{-4}x + 7.645 \times 10^{-3}$ ($R^2 = 0.997$)	14.2	12.0	91.8/92.9
acetylcodeine	342	1.0	20	2	40	40−4000	$3.890 \times 10^{-4}x + 2.784 \times 10^{-2}$ ($R^2 = 0.994$)	19.3	18.9	85.5/100.9
noscapine	413	0.25	5	0.5	10	10−2000	$4.614 \times 10^{-4}x + 1.669 \times 10^{-2}$ ($R^2 = 0.991$)	14.3	12.7	86.4/86.5
levallorphan[f]	284	0.5	10	1	20	20−4000				

[a] Concentration (ng) injected on-column. [b] Mean of eight calibration graphs obtained on 1 day. [c] Within-day reproducibility at LOQ, coefficient of variation (CV%), $n = 8$. [d] Between-day reproducibility at LOQ, coefficient of variation (CV%), $n = 8$. [e] Accuracy at 200/1000 ng/mL for papaverine and 200/2000 ng/mL for the other opium alkaloids. [f] Internal standard.

acetonitrile (50:50, v/v) and spiked with internal standard (100 ng oc) to perform quantitation in the linear range of the detected opium alkaloids.

Procedures. *Chromatographic Conditions.* Chromatographic separation was performed using a Chromolith Performance column (100 × 4.6 mm) kept at ambient temperature (Merck-Eurolab). The flow was optimized at 5 mL/min, and the following simple reversed-phase gradient program was employed: (A) water−acetonitrile (80:20, v/v) and (B) acetonitrile. The gradient profile started with 100% A, changed linearly to 79% A and 21% B in 1.5 min, stayed there for 1.5 min, again increased to 30% B in 1 min, and finally returned to 100% A in 1 min. Under these conditions, the last peak of interest, i.e., levallorphan, eluted after 4.4 min with an injection-to-injection cycle of maximum 7 min including reequilibration and reporting.

Mass Spectrometric Conditions. Due to the high flow, necessary to operate the HPLC system with the Chromolith Performance column, a postcolumn split (∼1/20 by volume), with a stainless steel tee splitter (0.25-mm bore, Vici AG, Valco Int,, Schenkon, Switzerland), was performed before the mobile phase reached the mass spectrometer. Nitrogen was used as the nebulizer gas at 400 kPa (supply pressure to the interface). High-purity helium was used as a buffer gas to trap ions in the mass analyzer at 260 kPa (supply pressure to the ion trap's capillary). The SSI parameters were set to the following optimized values: capillary voltage, 0 kV; drift, 70 V; focus, 35 V; cover plate temperature, 250 °C; aperture 1 temperature, 150 °C. Standard MS parameters were operated under the following conditions: accumulating masses, 200−350 amu; accumulation voltage, 0.075 V; low-mass cutoff, 100 amu; scan range, 100−450 amu.

Mass chromatograms of the molecular ions $[M + H]^+$ of the seven compounds and the internal standard were reconstructed at the m/z values given in Table 1. Under the above-described general MS conditions, one of the opium alkaloids, namely, noscapine, showed partial up-front fragmentation with the formation of the fragment ion m/z 220. The mass chromatogram for MS analysis was therefore reconstituted by the sum of the precursor and the fragment ion. Also, when solutions were kept for a longer period of time in the glass containers, sodiated molecular ions ($[M + Na]^+$) of the opium alkaloids appeared. Since each sodium adduct represents one original molecule, they were taken into account in MS analysis. A total ion chromatogram (TIC) and the mass chromatograms of the seven opium alkaloids and the internal standard, are shown in Figure 1.

Validation. The limits of detection and quantitation are defined as the concentrations for which a signal-to-noise ratio of 3−5 and, respectively, 10 are obtained.[22] The results obtained by the LC/SS-MS method for the opium alkaloids are summarized in Table 1. Linear dynamic range, regression equation (using minimum six calibrator points ranging from LOQ up to maximum 4000 ng/mL for each compound, $n = 8$), within- and between-day reproducibility ($n = 8$), and accuracy at 200 and 1000 ng/mL for papaverine and 200 and 2000 ng/mL for the other compounds of the opiate mixture are also summarized in Table 1. Finally, the method was applied to real-time seized heroin street samples in an effort to test the applicability of the analysis (see Figure 2.).

Safety Considerations. The method demands no specific safety precautions. General guidelines for work with organic solvents, acids, and bases have to be respected.

RESULTS AND DISCUSSION

Prior to the development of the LC/SS-MS method, we wanted to evaluate the SS ionization technique in more detail. Since sonic spray is a relatively novel ion source for LC/MS, developed in the beginning of the 1990s, it is not as well studied as the more traditional and commonly used pneumatically assisted electrospray or IS.[23] Both SS and IS, however, are based on nebulization or spray ionization processes, where the LC eluent is sprayed to form a mist of droplets which are charged by the nonuniformity of the ion concentrations in solution.[11] After solvent evaporation and continuous fission of the droplets, highly charged microdroplets and finally gas-phase ions are produced. Details of the latter process are not yet clearly understood, but two models for ion

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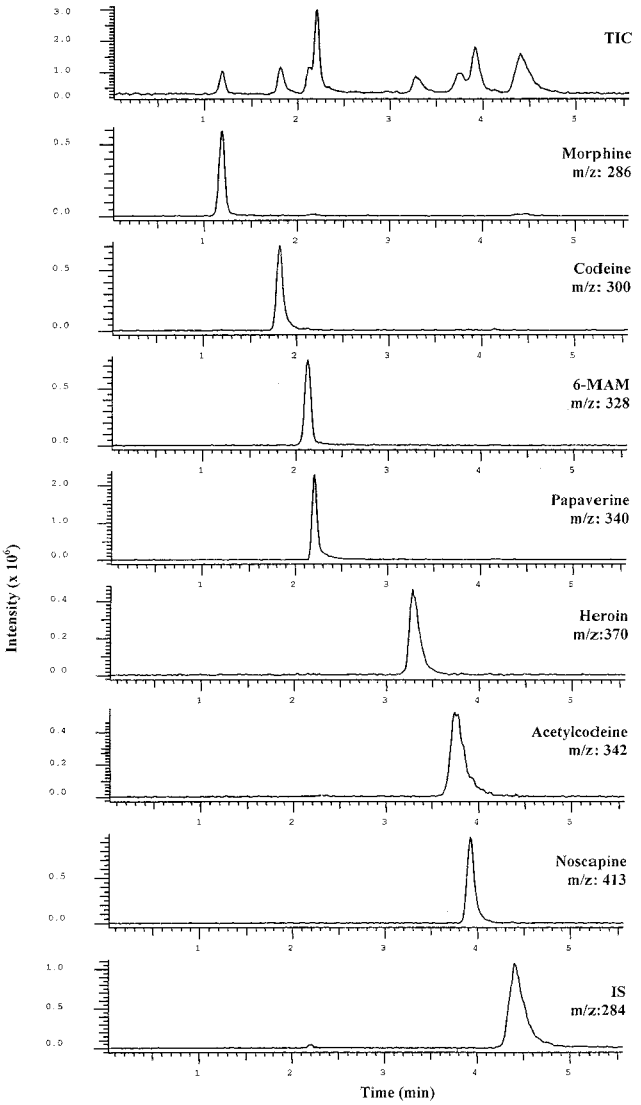

Figure 1. Typical total ion and selected ion chromatograms of a standard mixture (1000 ng/mL) of the opium alkaloids and the internal standard.

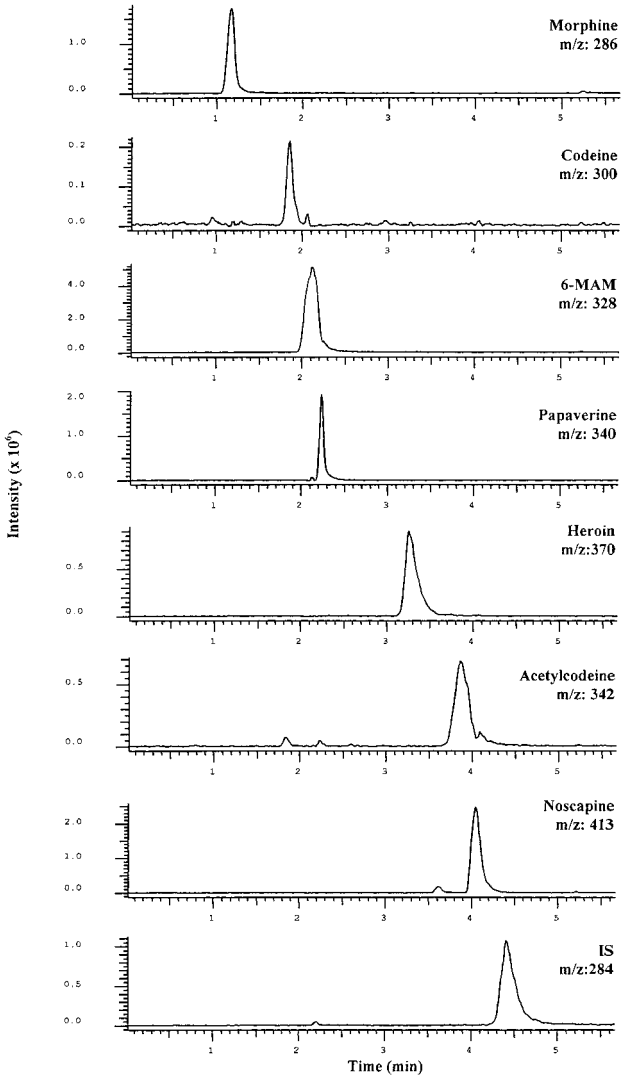

Figure 2. Selected ion chromatograms of an authentic heroin street sample.

formation have been proposed, namely, the ion evaporation model and the charge residue model as stated in the review of Kebarle and Tang.[24] The ion formation process, however, appears to be independent of the spray ionization technique while charged droplet formation is characteristic for each ion source and largely determines the ion formation efficiency.[11] Therefore, ionization in IS and SS is considered primarily a liquid-phase ionization technique, although, especially for smaller molecules, gas-phase ion−molecule reactions between ionized solvent and neutral analyte molecules should be taken into account as well.[25] Consequently, we were especially interested in the influence of the LC mobile-phase composition on the ionization efficiency of our target compound. In an effort to evaluate the similarities between both the IS and SS interfaces and possibly find explanations for the observed SS phenomena in the theory of the well-studied ES and IS interfaces, identical experiments were carried out on both ion sources and their behavior and performance was compared.

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To optimize the instrument (interface and MS) parameter settings, sample introduction by infusion was employed. Morphine was chosen as a model compound since it has the basic structure of a large number of opium alkaloids and their derivatives. A syringe pump (Harvard Apparatus, Hollington, MA) delivered a direct and continuous flow (25 μL/min) of the target compound (20 μg/mL morphine in water−methanol (50:50, v/v)) to the MS. To create realistic ionization conditions, the syringe pump was coupled in parallel to the HPLC gradient pump by a stainless steel tee piece. The gradient pump delivered a mixture of water−methanol (50:50, v/v) at a flow of 225 μL/min. This led to a total flow of 250 μL/min directed to the MS. The optimized SSI interface and MS parameters are given above in the Experimental Section. The IS interface parameter settings were as follows: auxiliary gas heater, 500 °C; capillary voltage, 3 kV; drift, 70 V; focus, 35 V; aperture 1 temperature, 150 °C; nitrogen gas flow, 300 kPa. Naturally the ion trap MS settings were kept identical for both interfaces. We immediately noticed that the obtained signal for morphine was much higher for SS than for IS, indicating a more extensive ionization in the first ion source (Figures 3−5). An explanation for this increased ion formation efficiency could

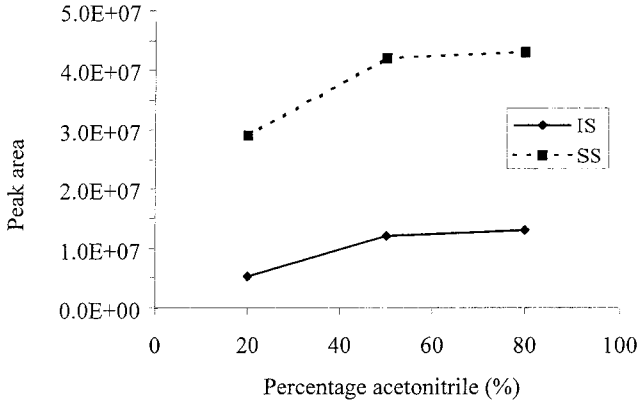

Figure 3. Influence of the percentage of acetonitrile in the LC mobile phase on the ionization efficiency of SS and IS (morphine as a model compound).

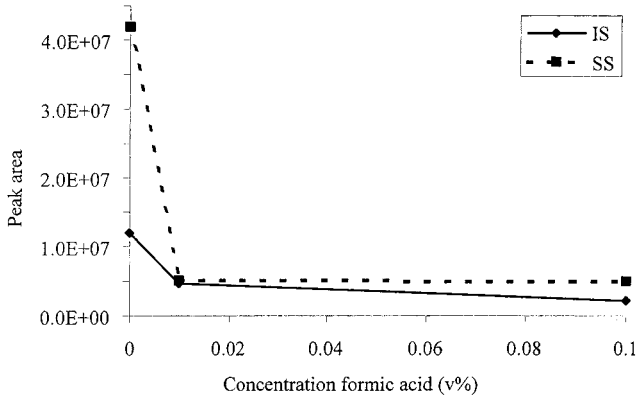

Figure 4. Effect of formic acid in the LC eluent on the ionization efficiency of SS and IS (morphine as a model compound).

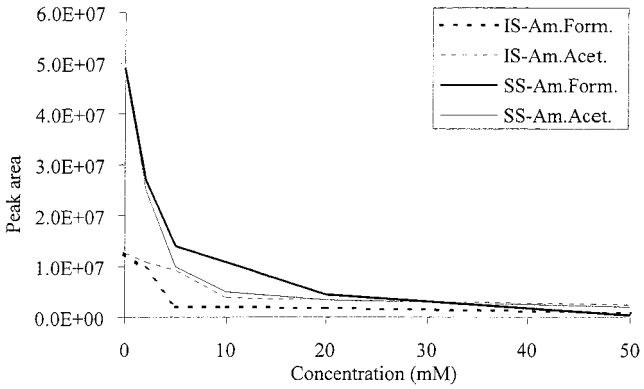

Figure 5. Influence of ammonium formate and acetate on the ionization of morphine with SS and IS (morphine as a model compound).

be found in the differences in the diameter of the droplet initially produced and in the different conditions for evaporation of solvent from charged droplets. In traditional ES, an external electric field induces nonuniformity of the ion concentrations inside the liquid front emerging from the spray capillary leading to the separation of charged droplets from the liquid front.[26] The diameter of the charged droplets initially produced has been measured to be ~1 μm, with a maximum distribution from 0.3 to 4 μm. In SS, droplets are produced by shear stress during pneumatic nebulization due to the high sonic gas velocity while nonuniformity of the ion concentration is supposed to be caused by the surface potential near the solution surface at the gas boundary.[11] Another mechanism for droplets charging, operative in both thermospray and sonic spray, is the statistical mechanism described by Dodd.[27] The initial droplet diameter is very similar to those droplets produced by ES, ranging from 0.5 to 5 μm. Finally, in IS, droplet formation is done by pneumatic nebulization while droplet charging is caused by an external electric field as in ES.[26] Due to the higher flow used for IS than for ES, this leads to initially charged droplets with a larger diameter, thus slower and less extensive microdroplet and gas-phase ion formation, which could be a reason for the difference in ionization efficiency.

From the literature it is known that opiates show a very diffuse fragmentation pattern with the formation of a large number of small fragments instead of one intense fragment ion.[28] The result is a drastic decrease in sensitivity when MS/MS analysis is performed in opiate analysis. Therefore, we decided at this stage of our research not to work in the MS/MS mode but to perform MS analysis. In the future, however, we will certainly investigate the applicability of this more selective type of analysis.

Subsequently, we evaluated the influence of the LC mobile-phase composition on the ionization efficiency of both interfaces for our target compound. The HPLC gradient pump was directly coupled to the MS through a Rheodyne manual injector. The mobile phase was delivered to the MS at a constant flow of 250 μL/min. A manual injection (5 μL) of a 20 μg/mL working solution of morphine, in the appropriate mobile phase, was performed in triplicate. An overview of the experimental parameters and of the results is given in Table 2.

In our first experiment, we investigated the influence of the organic modifier present in the mobile phase. First, we compared the ionization efficiency of a mixture of water−methanol and water−acetonitrile, both 50:50 by volume. For both interfaces, an increase of 50% in peak area was seen when acetonitrile was used as the organic modifier. Second, we changed the amount of organic solvent present. The following mixtures of water−acetonitrile were tested: 80:20, 50:50, and 20:80 (v/v). As can be seen in Figure 3, we noticed that the ionization decreased significantly when less than 50% acetonitrile was present. A higher percentage of organic solvent, however, did not result in a corresponding gain in ionization efficiency. This phenomenon was also observed for IS, and here it was thought to be due to the influence of a number of solvent characteristics on the formation of gas-phase ions with electrospray and ion spray, namely, solvent viscosity, solution conductivity, and solution surface tension.[26,29] When the amount of organic solvent in the mobile phase increases, the droplets initially formed at the tip of the capillary will decrease in size because of the decreasing viscosity and higher volatility of the eluent.[30] At the same time, the increasing solvent conductivity will result in a larger spray current while the decreasing surface tension of the droplet will lower the onset potential of the ion spray, i.e., the minimum voltage required to

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Table 2. Comparison of Ionization of Morphine by IS and SS (Positive Ion Mode) with Different Eluent Compositions

organic solvent	peak area IS ($\times 10^{-7}$) ($n = 3$)	peak area SS ($\times 10^{-7}$) ($n = 3$)
methanol−water (1:1)	0.8	2.8
acetonitrile−water (1:1)	1.2	4.2
acetonitrile (%)		
20	0.5	2.9
50	1.2	4.2
80	1.3	4.3
formic acid (vol %)		
0 v	1.2	4.2
0.01 v	0.5	0.5
0.1 v	0.2	0.5
ammonium formate (mM)		
0	1.2	4.7
2	1.0	2.7
5	0.2	1.5
10	0.2	1.2
20	0.2	0.5
50	0.1	0.1
ammonium acetate (mM)		
0	1.2	4.7
2	1.1	2.5
5	0.9	1.1
10	0.4	0.5
20	0.4	0.4
50	0.3	0.3

form the Taylor cone. The overall effect of the increasing amount of organic modifier was probably due to a combination of all the above-mentioned solvent characteristics. The difference between acetonitrile and methanol, seen for IS in our experiment, was mainly due to the lower viscosity of the water−acetonitrile mixture compared to the mixture water−methanol. Furthermore, parameters such as polarity, proton affinity, and gas basicity contribute to this effect as well.[31,32] Although in SS charged droplet formation is not performed by application of an external electrical field but by shear stress due to the sonic gas velocity, it is thought that the same solvent characteristics are the basis of the observed phenomena. Therefore, a similar effect for SS was observed, yet in a less extensive way.

In a next experiment, we evaluated the influence of a volatile acid in the mobile phase on the ionization of our target compound. In the literature, we found that formic acid was the most frequently used volatile acid in opiate analysis by LC/MS and this in concentrations up to 0.1 vol % for both ESI and IS.[14,15] Therefore, this was taken as our upper limit. Formic acid was tested in two concentrations: 0.01 and 0.1 vol % in water−acetonitrile (50:50, v/v). In Figure 4, it can be seen that, for SS as well as for IS, even a small amount of formic acid caused a drastic ionization suppression. From the theoretical basis of electrospray, it is assumed that the reason for this phenomenon could be a combination of a surface tension effect and ion pairing of cations and anions, which has already been reported for trifluoroacetic acid (TFA).[3] On one hand, the stability of the spray will be disrupted by a change in the conductivity. On the other hand, charge separation at the tip of the capillary might be less effective due to the increasing negative ion concentration, which might lead to a higher concentration of negative counterions in the droplet. The charge-to-mass ratio of the droplets generated will be lower, indicating that the droplet charging becomes less effective. Subsequent fission of the droplets will lead to a smaller amount of highly charged microdroplets, while the large parent droplets will act as dumps for the unwanted ion pairs.[24] Thus, the anion more or less masks the positive charge on the analyte molecule and thereby prohibits gas-phase ion formation. Since in SS droplets are also charged by a nonuniformity of ion concentrations in solution, a similar suppressive mechanism is expected.

In our final experiment, we evaluated the influence of buffer systems present in the LC eluent. Buffers are mostly added for chromatographic purposes; however, in some cases, it has been reported that they enhance the ionization as well.[33] In the literature, we found that the volatile buffers, ammonium formate and acetate, were mostly used in opiate analysis by LC/MS.[13,34] Therefore, we tested both ammonium buffers in the following concentrations: 2, 5, 10, 20, and 50 mM in water−acetonitrile (50:50, v/v). Since we experienced the catalyzing effect of ammonium ions on the hydrolysis of heroin in previous experiments, the pH of the mobile phase was always lowered to 4−4.5 with the appropriate acid, in an effort to overcome this problem.[2] The effects of increasing concentrations of both buffer systems on the ionization of morphine are shown in Figure 5. Even low concentrations of buffer in the LC mobile phase showed an extreme signal suppression for both interfaces. A partial explanation for this phenomenon might be the effect of the acid as previously discussed. However, not only will the presence of the acidic part of the buffer influence the ionization of our target compound, it was also thought that the ammonium ions behave as additional surface-active electrolytes, competing with the analyte molecules for ion emission at the droplet surface. Since the emission rate constant k from morphine is rather low, the presence of other electrolytes seems to have a serious impact on the ionization.[24,26] A combination the previous parameters is thought to be the reason for the severe signal suppression, in both IS and SS.

With the knowledge gained from these experiments, we concluded that a mixture of water and acetonitrile without addition of acids or buffer systems was the optimal mobile phase for the ionization of the opium alkaloids with the sonic spray interface. Therefore, a column with a high separation efficiency and performance was required. Furthermore, we wanted to increase the sample throughput by using so-called "high-speed" columns. To this end, the monolithic Chromolith Performance column (100 × 4.6 mm) proved to fulfill these demands. The column was kept at ambient temperature in the column oven, and the flow was optimized at 5 mL/min. The above-described simple reversed-phase gradient program based on water and acetonitrile was employed (see Procedures). Due to the limited flow rate acceptable for SS-MS, a connection between the LC system and the mass spectrometer was made by means of a postcolumn split of ∼1/20 (250 μL/min to the MS). A TIC and the mass chromatograms of the respective parent ions are shown in Figure 1. The method resulted in an analysis time of 5 min for each sample and a total

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injection-to-injection cycle of maximum 7 min including reequilibration and reporting. This indicated that a total of ∼200 samples could be analyzed per day, thus leading to a serious increase in sample throughput compared to more traditional opiate analysis.

Finally, the optimized method was validated. Validation parameters for each of the opium alkaloids are summarized in Table 1. The LODs and LOQs ranged from 2 to 20 and from 5 to 40 ng/mL, respectively. Although this was higher than in most other methods,[5−7] the sensitivity of the method was still significantly relevant for heroin impurity profiling purposes, where often highly concentrated samples are analyzed. Furthermore, the loss in sensitivity was more than compensated for by the gain in sample throughput. Correlation coefficients (R^2) greater than 0.990 were obtained for all compounds, fulfilling the analytical standard criteria. Within-day and between-day reproducibilities at the LOQ level were all below 20% except for morphine (between-day CV value of 20.3%). Accuracy, calculated at high- and low-quality control concentration ranged from 85.4 to 115.5%. The applicability of the method was demonstrated by the analysis of illicit heroin street samples. An example of a chromatogram obtained for a real-time heroin sample is shown in Figure 2. In future, the method will be applied for heroin impurity profiling purposes.[35]

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CONCLUSIONS

The performance of sonic spray ionization as an interface for LC/MS in opiate analysis proved to be very similar and in some cases even superior compared to the widely used ion spray. Therefore, it is thought that in the future this ion source will become more important and will take its place beside the already widely used API interfaces for LC/MS, namely, ESI (pneumatically assisted or not) and APCI.

Furthermore, the quadrupole ion trap proved to be an excellent mass analyzer to apply in opiate analysis. High sensitivity, reproducibility, and stability proved to be of good quality.

Finally, the use of a monolithic column allowed us to develop a fast method without any loss in separation efficiency. It is thought that the application of monolithic columns in LC/MS can be a very powerful tool for the future development of fast yet highly selective methods.

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