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Talanta 69 (2006) 795–799
Talanta
www.elsevier.com/locate/talanta

Quantitative analysis of morphine and noscapine using corona discharge ion mobility spectrometry with ammonia reagent gas

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Received 15 May 2005; received in revised form 9 October 2005; accepted 4 November 2005
Available online 15 December 2005

Abstract

Morphine and noscapine were determined using corona discharge ion mobility spectrometry. The detection limits were 5.6×10^{-11} and 6.7×10^{-11} g for morphine and noscapine, respectively. The linear dynamic ranges of the calibration plots for the compounds were about three orders of magnitude. The method has also been successfully applied for simultaneous determination of the compounds using the standard addition method.

Keywords: Corona discharge; Narcotics; Ion mobility spectrometry

1. Introduction

Noscapine (narcotine) as an opium-derived compound, is the second most abundant alkaloid in opium (2–8%), and is used as an index for the origin of heroin samples [1]. Narcotics are commonly used as therapeutic agents and some of these compounds are also frequently abused as illicit drugs [2]. Commercial opium is usually standardized to contain 10% morphine [3]. This compound plays an important role in the treatment of acute and chronic pain. Joshi [4] has discovered that noscapine and its analogs are effective anti-tumor drugs.

Different methods have been developed for the detection and determination of opiate derivatives, including GC–MS [5–7], HPLC [8–11], chemiluminescence [12,13], direct fluorimetric [14], capillary electrophoresis [15,16] and ion mobility spectrometry (IMS) [17,18].

Ion mobility spectrometry (IMS) is a well-known technique, which offers low detection limit, fast response, simplicity, and portability. The main advantages of IMS relative to other methods such as GC–MS are its simplicity and portability. Nowadays, miniaturized IMS has been constructed, enhancing its potential for using in security purposes. However, a separation technique is required in quantitative analysis of compounds in a mixture, before the sample is introduced into the IMS. Ionization source is one of the key parts of an IMS instrument. ^{63}Ni is a common ionization source for IMS. We have investigated the capability of continuous corona discharge as an ionization source for IMS in both positive [19,20] and negative modes [21]. The advantage of corona discharge as an ionization source over ^{63}Ni is a higher total ion current by about an order of magnitude. This results in a better sensitivity, a higher signal to noise and a wider working range. The higher current also allows using narrower ion pulse widths, which enhances the resolving power. In addition, there is no need to use radioactive materials.

The IMS works in positive and negative modes. In the positive mode of IMS, the analyte is ionized in ion-molecular exchange reactions with reactant ions, which are commonly hydrated protons. Other reactant ions such as ammonium and halides have also been used as alternative reagent ions for enhancing sensitivity and selectivity of the method [22]. In this work, the application of corona discharge IMS for analysis of morphine and noscapine by using ammonia as the reagent gas is investigated.

2. Experimental

2.1. Apparatus

The IMS apparatus with the continuous corona discharge as ionization source has been described previously [20]. The main

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doi:10.1016/j.talanta.2005.11.016

Table 1
IMS operation parameters

Parameter	Setting
Corona voltage	2.00 KV
Drift field	436 V cm^{-1}
Drift gas flow (N_2)	600 ml min^{-1}
Carrier gas flow (N_2)	250 ml min^{-1}
Drift tube temperature	180 °C
Injection temperature	230 °C
Drift tube length	11 cm
Reactant gas	NH_3
Shutter grid pulse	100 μs

parts of the instrument are: the IMS cell, the needle for producing the corona, two high voltage power supplies, a pulse generator, an analog to digital converter and a computer. In this work, the glass cell of the system was substituted with 16 aluminum rings of 4.6 cm internal diameters, which were separated from each others by thin PTFE rings. The aluminum rings are connected by a series of resistors to form the electric field gradient. The corona electrode is a sharp needle, made of stainless steel, inserted into a Teflon piece and it is fixed at one end of the cell. The shutter grid is made of two series of parallel wires biased to a potential, creating an orthogonal field relative to the drift field, to block ion passage to the drift tube. The grid potential is removed for a short period of time by the pulse generator, to admit an ion pulse to the drift region. Generally, this period of time was selected 100 μs. The sample introduction system was the same as the previous work [23]. In brief, 10 μL of the sample was placed on the filament mounted on a cap and after evaporating the solvent, the cap was inserted into the injection port. The analyte is carried into the IMS cell by the carrier gas. A small capsule containing ammonium carbamate was used for doping the carrier gas with ammonia. The amount of the NH_3 doped into the carrier gas was controlled by a diffusion barrier. Then, the gas was passed through a 13X molecular sieves (Fluka) trap to remove water vapor and other possible contaminations before entering into the IMS cell. The optimized experimental conditions for obtaining the ion mobility spectra of the compounds are listed in Table 1.

2.2. Chemicals and solutions

Morphine and noscapine were obtained from Temad Co. (Tehran). Stock standard solutions (200 μg mL^{-1}) of these compounds were prepared in methanol (Merck). Working solutions (0.01–150 μg mL^{-1}) were prepared by successive dilution of the stock solutions.

3. Results and discussion

Ion mobility spectra of morphine, noscapine and background are shown in Fig. 1. Ammonium ion is the reactant ion, which can be distinguished in the blank spectrum. The high proton affinity of ammonia with respect to water is helpful in eliminating the ion clusters of $(H_2O)_nH^+$. The morphine spectrum shows two peaks, M_1 and M_2, with reduced mobility values (K_o) of (1.33 ± 0.08) and (1.29 ± 0.07) cm^2V^{-1} s^{-1}, respectively. These values are

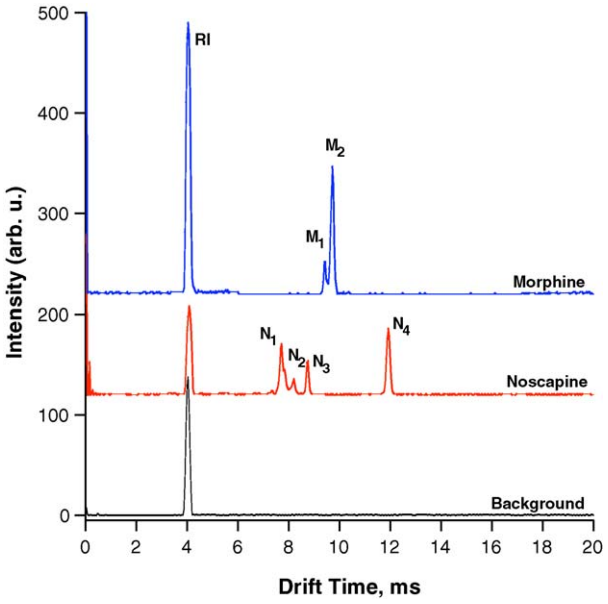


Fig. 1. The ion mobility spectra of the morphine, noscapine and background.

very close to the reduced mobility of $[M–(H_2O)]^+$ (1.26) and M^+ (1.22) reported by Lawrence [18], respectively. Based on his work it might be concluded that the first and the second peaks in morphine spectrum are $[M–(H_2O)]^+$ and M^+, respectively. The noscapine spectrum shows four peaks, N_1, N_2, N_3 and N_4, with K_o of 1.65, 1.51, 1.42 and 1.04 cm^2V^{-1} s^{-1}, respectively. There is not any reported reduced mobility for product ions originated from noscapine in the literature. Therefore, the chemical formula of the ions have not been recognized. The reduced mobilities of the ions together with their corresponding standard deviations are listed in Table 2.

The reasons for observing two ion peaks for morphine and four ion peaks for noscapine can be attributed to their chemical structures, which are shown in Fig. 2. According to this figure, it can be observed that the morphine has a more stable structure than that of noscapine. The ion mobility spectrum of morphine shows only two peaks (M^+ and $[M–(H_2O)]^+$), which are originated from mother molecule. However, noscapine has the σ-bond between the phthalidyl moiety and tetrahydroisoquinoline, which can be broken and consequently different fragments of noscapine might be formed. The product ions might be produced from proton transfer, cluster formation, dimerization and formation of clustered dimmer [24].

Table 2
Characteristic ions for morphine and noscapine ion mobility spectra

Peak	Reduced mobility, K_o (cm^2V^{-1} s^{-1})	Drift time, t (ms)
RI (NH_4^+)	3.20 ± 0.11	3.92
M1	1.33 ± 0.08	9.46
M2	1.29 ± 0.07	9.76
N1	1.65 ± 0.09	7.62
N2	1.51 ± 0.08	8.32
N3	1.42 ± 0.06	8.86
N4	1.04 ± 0.04	12.02

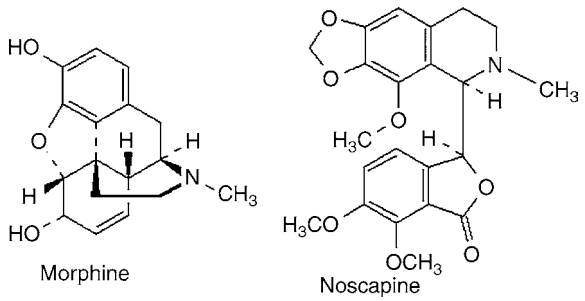
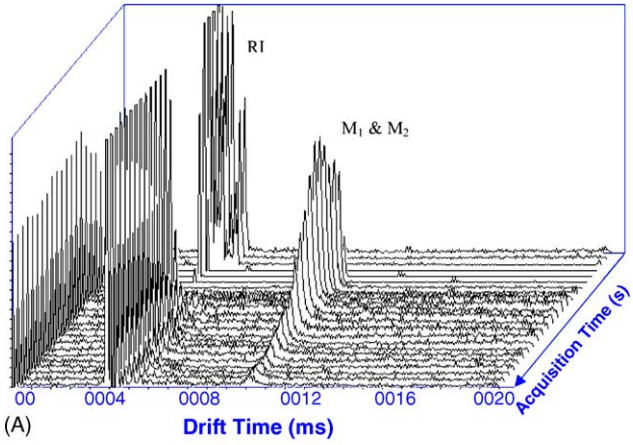


Fig. 2. The chemical structures of narcotic compounds studied.

3.1. Quantitative analysis

3.1.1. Response of the analyte

The watershed 3D plots of the ion mobility spectra of morphine and noscapine with concentrations of 5.0 and 5.0 μg ml^{-1}, are shown, in Fig. 3A and B, respectively. The figures show that the relative peak intensities are changed during the acquisition time (from the injection time until the sample peaks disappear). The product ion peaks appear after a short period of time, reach a maximum and decay almost exponentially. The additional peaks close to RI in Fig. 3A relative to Fig. 1 are originated from of the



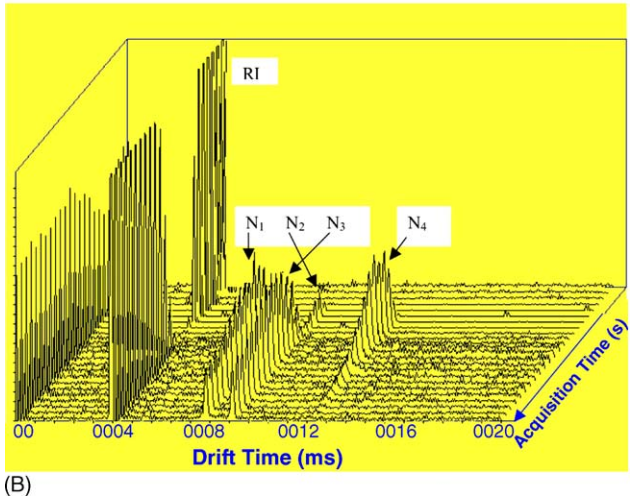


Fig. 3. The section of the watershed 3D plots of the ion mobility spectra of (A) morphine and (B) noscapine.

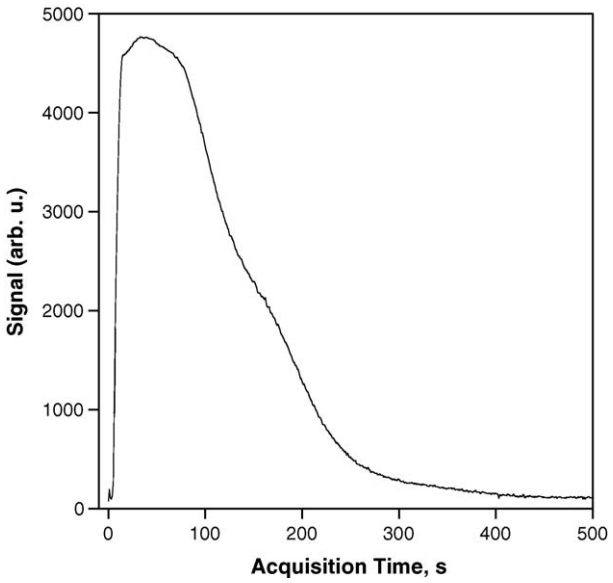


Fig. 4. The sum of the areas of peaks (total) against the acquisition time obtained by exposure to amount of 2.0 × 10^{-8} g of morphine.

reactant ions $(H_2O)_nH^+$. These ions remain when the ammonia concentration in the cell is not high enough to react with all the proton cluster ions.

The sum of the total peak areas for morphine and noscapine are plotted against the acquisition time. This plot is shown in Fig. 4 for injection of 20 ng morphine. The integration of each curve is considered as the response for the corresponding compound with respect to the injected amount.

3.1.2. Analytical parameter

The calibration curves for morphine and noscapine were obtained by plotting their respective responses against the amount of each compound. Working and linear dynamic ranges for morphine and noscapine are shown in Fig. 5. As can be seen, the working range is about four orders of magnitude and the linear dynamic range is about three orders of magnitude for both compounds. The detection limits were 5.6 × 10^{-11} and 6.7 × 10^{-11} g for morphine and noscapine, respectively. The analytical parameters of the corona discharge IMS method for determination of morphine and noscapine are given in Table 3. In comparing the detection limits and dynamic range of the proposed method with other analytical methods, the GC-tandem MS method [5] has the lowest detection limit of 5.7 pg ml^{-1} for mor-

Table 3
Analytical parameters for morphine and noscapine using conrona discharge-IMS

Parameter	Morphine	Noscapine
Linear range (g)	1.0 × 10^{-10}–1.0 × 10^{-7}	5.0 × 10^{-10}–2.0 × 10^{-7}
Correlation coefficient, R^2	0.9964	0.9949
Detection limit (g)	5.6 × 10^{-11}	6.7 × 10^{-11}
Relative standard deviation, R.S.D. (%) for 50 ng and n = 5	11.5	13.1

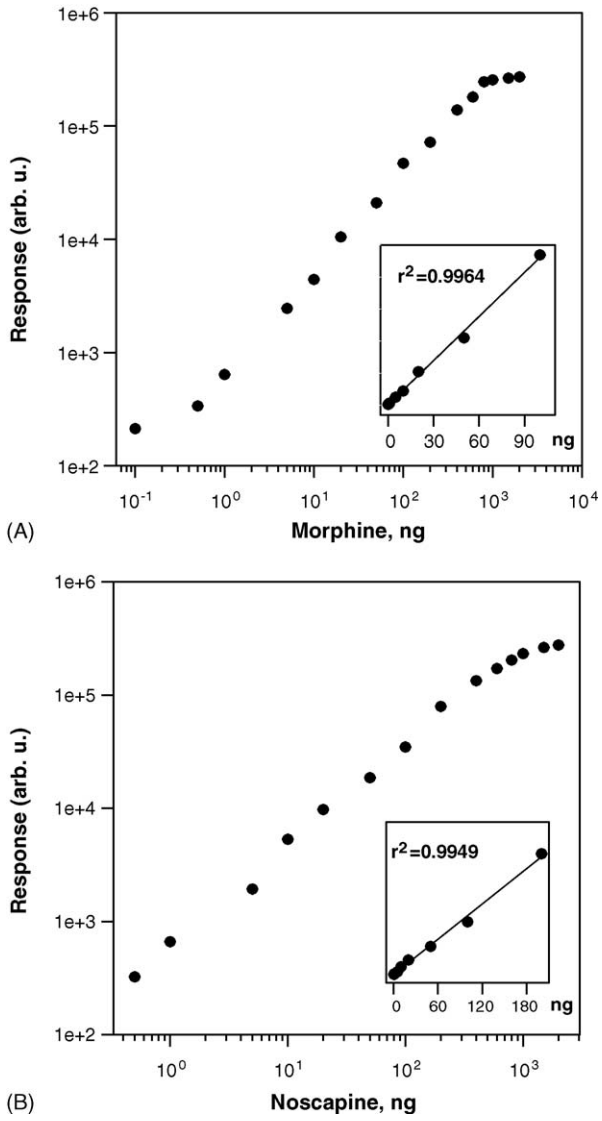


Fig. 5. Plots of IMS response against the amount of (A) morphine and (B) noscapine, the plots shown as onset of these graphs show the linear section of the calibration curves.

phine with a dynamic range of about three orders of magnitude. However, the method is very expensive and highly sophisticated. In addition, morphine must be derivitized with pentafluoro-1-propanol in order to make it suitable for the measurement. A few of the methods have lower detection [10,14], but the proposed method presents superior detection limits with respect to several other methods [7,8,11,15,16]. The linear dynamic range, two to three orders of magnitude, is comparable with the mentioned methods.

3.2. Analysis of morphine and noscapine in mixtures

Ion mobility spectrum of a mixture of morphine and noscapine is shown in Fig. 6. Comparison of this spectrum with the individual spectra of morphine and noscapine shows that the ionic peaks of morphine and noscapine do not overlap with each other. This is promising for selecting a suitable method for simultaneous determination of these compounds. However,

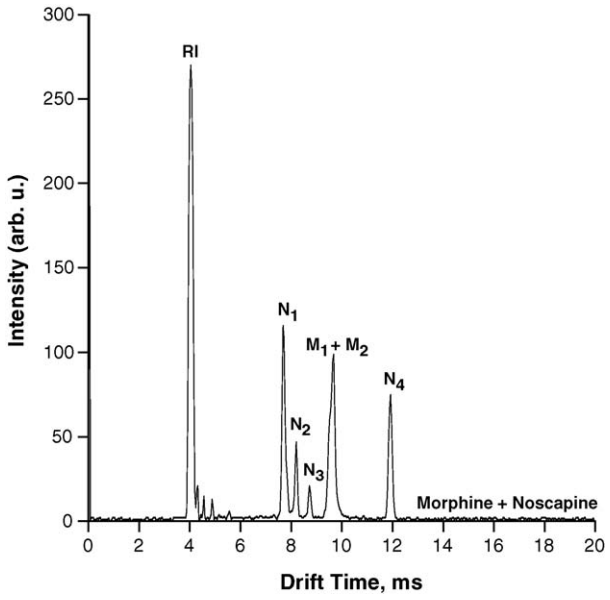


Fig. 6. The ion mobility spectrum of a mixture of morphine and noscapine.

the influence of sample matrix should also be investigated. In order to check this effect, the slope of the calibration curve for a set of standard morphine solutions was compared with the slope of the calibration curve for the same set of standard morphine solutions in the presence of a constant amount of noscapine. The same comparison was also performed for the calibration curves with noscapine in the presence or absence of morphine. The calibration curves are shown in Fig. 7 and the statistical results for comparison of the slopes are given in Table 4. The columns in Table 4 are the slopes and their standard deviations, F values for comparison of two variances, the pooled estimated standard deviations, the calculated t values and the tabulated t values, respectively [25]. It can be observed that the slopes of the calibration curves for the mixtures are not in the confidence interval for the slopes of the calibration curves for the single compounds at 95% confidence level and the calculated t values are larger than the tabulated t values. Therefore, it can be concluded that the slopes are significantly different, i.e., noscapine imposes a matrix effect on morphine signal and vice versa.

Eiceman [24] has described different methods for eliminating matrix effects in IMS. In this work, standard addition method was used to cope with the matrix effect. Standard solutions with different concentrations of morphine and noscapine were prepared and their concentrations were predicted using standard addition method. The recovery percentages for the compounds are listed in Table 5. The results show that simultaneous deter-

Table 4
Statistical results for comparison slope of the calibration curves

Graph	Slope	$F_{exp.}$ (S_A^2/S_B^2)	$F_{tab.\ (0.05);\ 3,3}$	S_{pooled}	$t_{exp.}$	$t_{tab.\ (0.05)}$
A_1	4572 ± 247	–	–	–	–	–
B_1	2949 ± 162	2.31	15.44	656	4.10	2.78
A_2	5288 ± 257	–	–	–	–	–
B_2	4071 ± 153	2.83	15.44	663	5.53	2.78

Table 5
Recovery percentages of morphine and noscapine for synthesized samples

Synthesis sample	Morphine (μg/ml)	Noscapine (μg/ml)	Morphine found (μg/ml)	Noscapine found (μg/ml)	Recovery (%) of morphine	Recovery (%) of noscapine
1	2.0	4.0	1.77 ± 0.38	3.75 ± 0.37	88.5	93.8
2	3.0	3.0	3.24 ± 0.31	3.37 ± 0.30	108.0	112.3
3	4.0	2.0	3.45 ± 0.28	1.98 ± 0.26	86.2	99.0

mination of morphine and noscapine using standard addition method is possible and the recovery percentages of the compounds validate the ability of the method.

It would be emphasized that generally, matrix effects have a diminishing influence on the selectivity of IMS. Therefore, a separation technique is required in quantitative analysis of compounds in a mixture before the sample is introduced into the IMS. For example in quantitative analysis of morphine and noscapine in biological samples such as urine and blood, first of all morphine and noscapine should be separated from the matrix and then the standard addition method is used to deal with the matrix effect imposes by noscapine on morphine signal and vice versa.

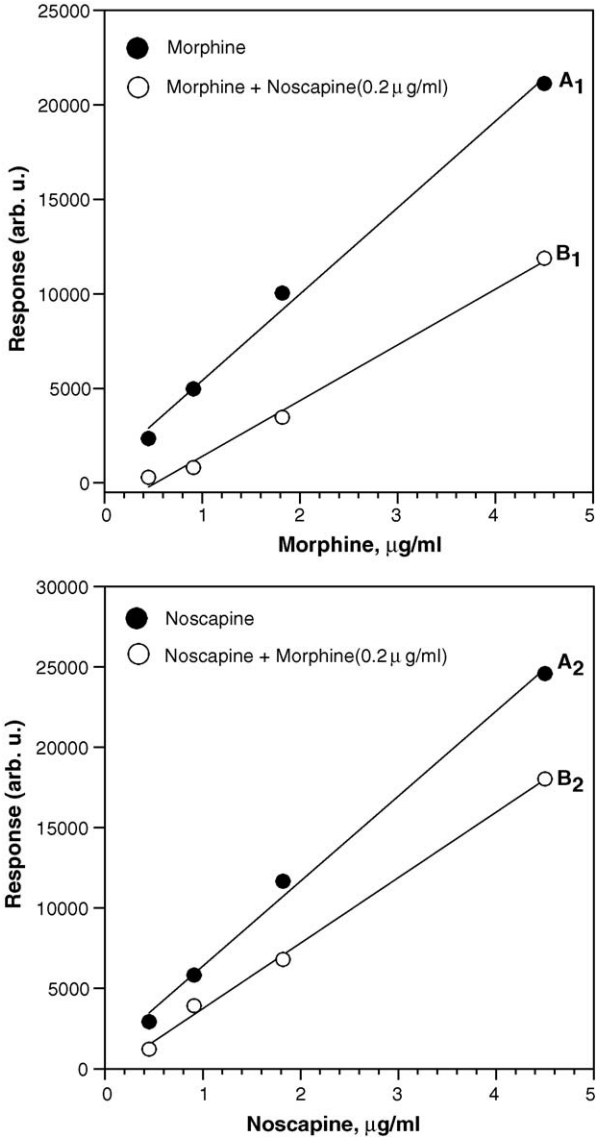


Fig. 7. The calibration curves of morphine, morphine + noscapine (upper) and noscapine, noscapine + morphine (lower).

4. Conclusions

Corona discharge-IMS has been used as an alternative method for the determination of morphine and noscapine. Low detection limits and wide dynamic ranges of the compounds reveal the capability of the method. In addition, simultaneous determination of morphine and noscapine can be performed using a simple standard addition method.

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