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Short communication

Determination of trace impurities of peptides and alkaloids by capillary electrophoresis–ion spray mass spectrometry

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Abstract

Two different mixtures have been analyzed by CE–UV–MS using selected ion monitoring (SIM) conditions to evaluate whether this technique can detect trace impurities in such mixtures. The first mixture consisted of two bioactive peptide analogues which included Lys–bradykinin (kallidin) and Met–Lys–bradykinin. The presence of 0.1% Lys–bradykinin was detected by SIM CE–MS but not by CE–UV at the 0.1% level as it migrated from the capillary column prior to the major component, Met–Lys–bradykinin. The second mixture consisted of two antibacterial alkaloids, berberine and palmatine. The presence of 0.15% palmatine was detected by CE–UV and SIM CE–MS at the 0.15% level as it migrated from the capillary column following the major component, berberine. These results suggest that SIM CE–MS offers the necessary separation efficiencies and sensitivity to provide a complementary analytical determination of trace components in such sample mixtures.

1. Introduction

Capillary electrophoresis–mass spectrometry (CE–MS) offers a promising technique for biomedical and biochemical studies to characterize biomolecules and other compounds of interest [1–7]. In the chemical, pharmaceutical and biotechnology industries there frequently is a need for the characterization of trace impurities in products or product formulations. To deal with these needs reports have appeared from several bioanalytical investigators who have attempted to characterize the impurities in peptides as well as drugs and drug products. These include a report of the determination of a 0.1% impurity in a synthetic peptide [8], a 0.075% impurity in a nicardipine drug substance [9], a 0.1% drug steroid impurity [10] and a 1% anticancer drug minor impurity [11]. A recent report using CE–MS described the determination of an impurity in biological matrices where the synthetic peptide impurity was identified and quantitated at the 0.7% level [12]. This report prompted us to further explore the analytical utility of CE–MS for the determination of trace level impurities in biological samples.

In this CE–MS investigation, two bioactive peptide analogues, Lys–bradykinin (kallidin) and Met–Lys–bradykinin, which are important compounds for dealing with pain as well as inflammation, were detected by CE–UV–MS under selected ion monitoring (SIM) conditions.

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Moreover, SIM CE–MS was also used to monitor trace levels of one antibacterial isoquinoline alkaloid, palmatine, in the presence of the other, berberine. These compounds are found at low levels in the bark of *Phellodendron chinese Schneid.* [13,14]. Comparisons of different relative levels of Lys–bradykinin and Met–Lys–bradykinin as well as berberine and palmatine were explored by CE–UV–MS to evaluate the potential of CE–MS to detect a trace impurity in such sample mixtures.

2. Material and methods

2.1. Chemicals

Bradykinin (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg, $M_r = 1060.2$), Lys–bradykinin ($M_r = 1188.4$, Met–Lys–bradykinin ($M_r = 1319.6$), Ile–Ser–bradykinin ($M_r = 1260.5$), berberine ($M_r = 336.4$) and palmatine ($M_r = 352.4$) were purchased from Sigma (St. Louis, MO, USA). All solvents, buffers and common chemicals were reagent grade or better and purchased from Fisher Scientific (Rochester, NY, USA).

2.2. Conditions for ion spray mass spectrometry

A Sciex TAGA 6000E atmospheric pressure ionization (API) triple quadrupole mass spectrometer (Thornhill, Canada) updated to an API-III with a scan range from m/z 10–2400 was used for all experiments. The ion spray sprayer was positioned approximately 1 cm off-axis and 1 cm away from the ion-sampling orifice and maintained at 4.5 kV with a flow of liquid nitrogen blow-off nebulizing gas maintained at 45 p.s.i. (1 p.s.i. = 6894.76 Pa). Polypropylene glycol in acetonitrile–water (80:20) (3 m*M* NH_4OAc) was used for tuning and mass-axis calibration for each mass-resolving quadrupole (Q1 and Q3). Electropherograms for peptides and alkaloids were acquired at a declustering energy of 60 V and 30 V, respectively. All CE–MS experiments were carried out in the SIM mode using the standard PE-Sciex Macintosh-based software.

2.3. Conditions for CE–MS

A high-performance CE system (Model P/ACE 2050, Beckman Instruments, Palo Alto, CA, USA) was used in this study. Separation was performed on an uncoated 120 cm × 50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

On-line UV detection (200 nm for the peptides and 254 nm for the alkaloids) occurred approximately 17 cm from the inlet of the CE capillary. The in-house ion spray interface shown in Fig. 1 consists of three concentric capillaries for introducing a sheath liquid flow and nebulizing gas. The sheath liquid flow was comprised of 80% CH_3CN and 20% 5 m*M* NH_4OAc at pH 3.5 and was delivered at 2 μl/min by an infusion pump (Harvard Apparatus, South Natick, MA, USA). The three concentric capillaries are coupled with the two stainless-steel Tee's [SGE, 1/32 in. (1 in. = 2.54 cm), see Fig. 1] for separate but simultaneous introduction of the sheath liquid flow and the nitrogen nebulizing gas. This device affords a robust CE–MS combination coupled with the ion spray interface. When the concentric capillaries are appropriately positioned at the tip as shown in Fig. 1 and the flow of sheath liquid and nitrogen nebulizing gas are optimized, the described experiments may be routinely performed.

The CE separation was accomplished by applying 30 kV at the anode end while 4.5 kV was applied to the cathode via the ion spray interface high-voltage supply resulting in a potential difference across the CE capillary of 25.5 kV. Samples were loaded into the anode end of the capillary via either a 5-s pressure injection or a 10-s electrokinetic injection at 10 kV. The pressure injection technique was used for 1 mg/ml samples of the peptides, while the electrokinetic injection technique was used for the μg/ml samples of the alkaloids.

All the solutions including the 25 m*M* NH_4OAc at pH 3.5 for peptides and 100 m*M* NH_4OAc at pH 4.5 for alkaloids were prepared fresh daily and filtered through 0.2-μm nylon HPLC syringe filters (Krackler Scientific, Albany, NY, USA) before use.

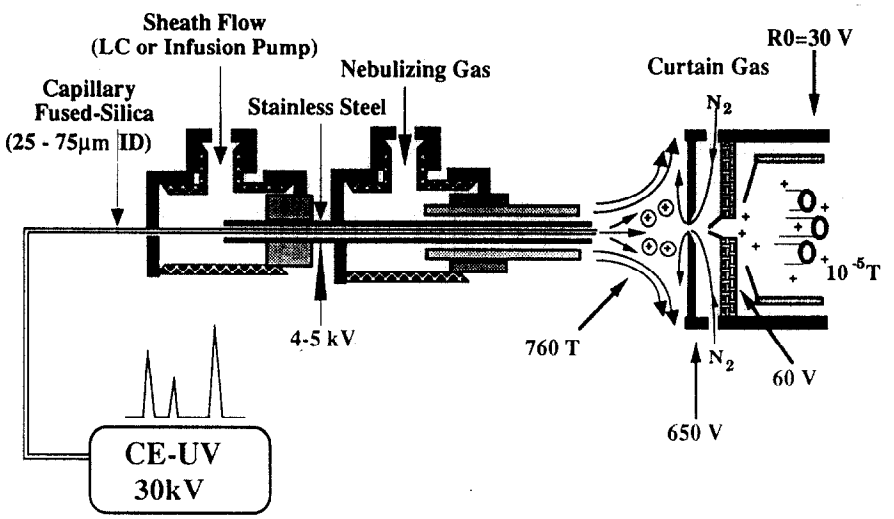

Fig. 1. Ion spray CE–MS interface equipped with a coaxial sheath-flow of liquid.

3. Results and discussion

The goal of this work was to determine the practical CE–UV–MS detection limits for representative minor components in simple synthetic mixtures. Two synthetic mixtures were prepared for study. One was a binary peptide mixture where the faster migrating peptide's concentration was systematically reduced relative to the other. This example was chosen to represent an impurity determination where the minor component migrates from the capillary exit before the major component. The second synthetic mixture was prepared containing berberine and palmatine with reduced amounts of the latter, longer-migration-time component. The minor component was monitored by CE–UV–MS as the concentration of this component was reduced in stages to 0.15% that of the major component. In each case CE–MS was conducted under SIM conditions where the abundant protonated molecule ion was monitored.

The corresponding doubly charged ions for the two peptides chosen for this study, Lys–bradykinin and Met–Lys–bradykinin (m/z 595 and 661, respectively) were monitored by SIM following their separation on an uncoated 50 μm I.D. capillary fused-silica capillary column (Fig. 2). Lys–bradykinin and Met–Lys–bradykinin were selected as a two-component mixture with lower levels of the former in the presence of the other. Lys–bradykinin was used as the "impurity" or diminutive component in the presence of higher levels of Met–Lys–bradykinin. Fig. 2 shows the CE–UV–MS comparison UV and SIM electropherograms for Lys–bradykinin and Met–Lys–bradykinin at ratios of 35, 5 and 0.1% by on-line CE–UV–MS. The Lys–bradykinin component present at the 35 and 5% levels in the binary mixture was readily detected by both UV and MS detection (see Fig. 2A and B). Although the peak heights in Fig. 2A appear comparable, the peak areas are indicative of the 35% level of the Lys–bradykinin component. It is interesting to note, however, that the 0.1% level of Lys–bradykinin is not observed in the CE–UV electropherogram (Fig. 2C, inset) although it is readily detected in the corresponding CE–MS electropherogram (Fig. 2C). This is in part due to the improved separation afforded in the case of CE–MS detection where the mixture components transit the entire 120 cm of the separation capillary prior to detection (see above). These results demonstrate that CE–MS can be a complimentary technique to UV for detecting the presence of trace levels of selected components. By CE–MS, however, one has an increased level of specificity due to the ability to

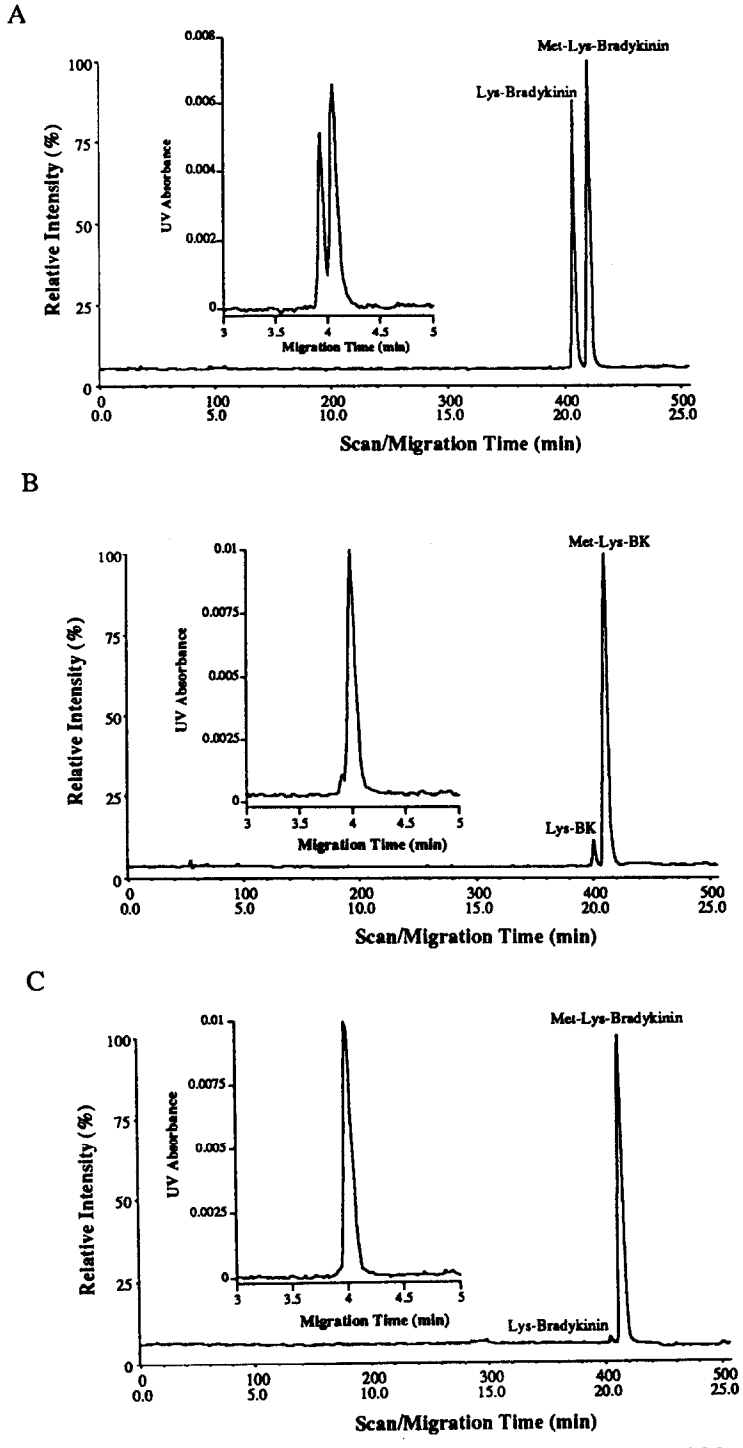

Fig. 2. SIM CE–MS electropherograms for a synthetic mixture containing Lys–bradykinin and Met–Lys–bradykinin with (A) 35%, (B) 5% and (C) 0.1% Lys–bradykinin in the presence of Met–Lys–bradykinin. BK = Bradykinin.

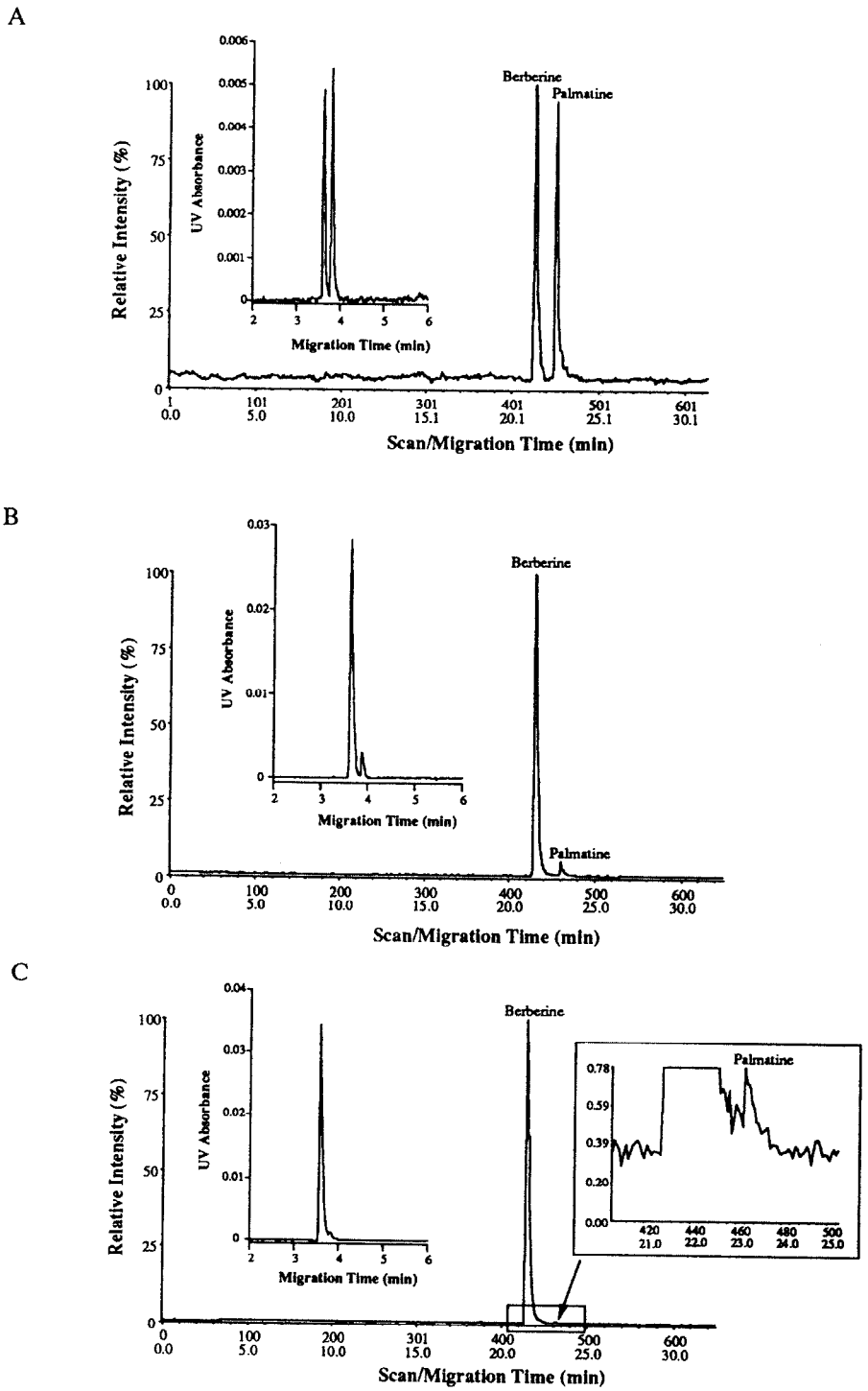

Fig. 3. SIM CE–MS electropherograms for a synthetic mixture containing palmatine and berberine with (A) 50%, (B) 4% and (C) 0.15% of palmatine in the presence of berberine.

monitor one or more ions characteristic of the target analyte(s). Of course if one has sufficient sensitivity it may be possible to acquire the full-scan mass spectrum for an impurity to facilitate its characterization in the event that it is an unknown compound.

The second example of combined CE–UV–MS determination of impurities is shown in Fig. 3. In this case the longer-migration-time component was chosen as the "impurity" in a binary mixture composed of berberine and palmatine. The percentages of palmatine in berberine in Fig. 3 were 50, 4 and 0.15%. It should be noted that both the UV and SIM electropherograms in these experiments reveal the presence of the trace component at each level. CE–MS detection of the minor component at the 0.15% level may be facilitated by amplification in the *y*-direction of the appropriate region in the electropherogram (Fig. 3C). As indicated above the added specificity benefits of CE–MS include monitoring ions that are characteristic of the target impurity. Full-scan mass spectra should also be especially helpful for characterizing unknown impurities in mixtures.

In conclusion, the combination of high sensitivity and specificity afforded by CE–MS demonstrated by these studies may provide complementary information for detecting and characterizing diminutive components in synthetic drug mixtures, drug metabolism profiles, byproducts of manufacturing and enantiomeric drug products. Recent results from this laboratory suggest that the determination of chiral mixtures by CE–MS may provide a new approach to validating the purity of enantiomeric drugs. Results from these studies will be published subsequently.

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