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The effect of liquid chromatography eluents and additives on the positive ion responses of cocaine, benzoylecgonine, and ecgonine methyl ester using electrospray ionization

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Abstract

We investigated the effect of common chromatography eluents and additives on the positive ion responses of ecgonine methyl ester (EME), benzoylecgonine (BZE), and cocaine (COC) using electrospray ionization (ESI). Primarily $[M + H]^+$ ions were observed, although decomposition of EME and COC to ecgonidine methyl ester gave a sizable peak at *m*/*z* 182.0. The results showed that the sensitivity for the test analytes was greatest in a mobile-phase consisting of a 1:1 mixture of 60% acetonitrile/40% acetone:100 mM ammonium acetate. There was no evidence of a correlation between sensitivity of $[M + H]^+$ ions and solution pH. Adducts derived from addition of ammonium salts and ammonium hydroxide, along with cluster ions were not observed, although cationization did occur for BZE (<1.0–23%). Signal intensities for COC (pK_a = 8.61) obtained under acidic conditions (pH = 2.55–2.80) and basic conditions (pH = 9.19–10.02) did not vary, suggesting that mechanisms other than in-solution ionization maybe key in formation of ions by the electrospray process.

Keywords: API/MS; Ecgonine methyl ester; Benzoylecgonine; Cocaine; LC

1. Introduction

Atmospheric pressure ionization mass spectrometry (API/MS) has gained widespread popularity as an analytical tool for the quantitative determination and structural characterization of pharmacologically active compounds in biological matrices [1,2]. The high sensitivity and selectivity provided by API when coupled to liquid chromatography/tandem mass spectrometry (LC/MS/MS) has reduced the time required for method development and sample analysis of drugs and their metabolites in biological matrices [3,4]. API is characterized by two ionization techniques ideally suited for analyzing small molecules: atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) [5–7]. Fundamentally, these two

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processes are distinguished by differences in ion generation, although considerable overlap exists in their range of applicability.

APCI is used routinely for high throughput and high sensitivity quantitative analysis of low polarity compounds. The APCI process generally produces protonated or deprotonated molecular ions, primarily via proton transfer (*positive ions*) or proton abstraction (*negative ions*) mechanisms. The sample is vaporized in a heated nebulizer before emerging into a plasma, which is formed within the atmospheric source and corona discharge needle. The extent of sample ionization is driven by the gas-phase proton affinities (PA) of all chemical species present in the APCI source. Efficient ionization is obtained in the positive mode when the analyte proton affinity is higher than that of the mobile-phase and endogenous source components.

ESI is a soft ionization process used to generate gaseous ionized species from liquid solutions. Ionization is believed to occur "in-solution" through production of a fine liquid spray in the presence of a strong electrical field [8–10]. The sample solution is sprayed from a region of high electrical intensity, at ~4000 V, where the highly charged droplets become electrostatically attracted to the orifice inlet of the mass spectrometer. Prior to entry into the mass spectrometer, dry gas, heat, or combination of the two are applied to the droplets to effect ion desolvation.

Two models have been proposed for the ion generation process: charge residue model (CRM) and ion evaporation model (IEM). In CRM, as the droplets condense, the electric field density on the droplet surface increases. The surface area of the droplet continues to decrease until the repulsive forces of "like-charges" on the surface exceed the droplet's surface tension. Subsequently, ions are ejected from the droplet through what is known as the "Rayleigh Limit" is reached [11,12]. The IEM model is similar to CRM in that, charge density also increases as the solvent evaporates. However, in this model coulombic forces overcome the adhesive force of the charged species on the surface, expelling ions directly from the surface into the gas-phase [13].

Factors that may effect generation of ions by API include: pH, pK_a, temperature, mobile-phase additives, flow-rate, solvent composition, and concentrations of electrolytes and analytes [14–16]. The importance of the addition of organic modifier on electrospray ion current stability, sensitivity, and performance has been examined [17–19]. Electrospray sensitivity gains achieved when employing various organic solvents has been investigated, showing that the higher the organic concentration in the solvent system, the greater the electrospray response [15]. Reports delineating the effects of solution pH and analyte pK_a on the response of protonated analyte molecules have been made, suggesting that pH might be a factor used to optimize a mixture for a particular analyte [14,20,21]. Also, the significance of various solution phase factors, such as viscosity, surface tension, and analyte characteristics on ESI response have been extensively examined [22,23].

ESI method development can be a time-consuming endeavor. Many conventional buffering and additive agents are deleterious to the electrospray ion generation process. As such, the ionization efficiency of a particular analyte can be affected by an ensuing competition for charge between all species present in the eluent [24]. In other experiments, the effects of electrolyte concentration on analyte response using ESI have been reported [16,24]. The analyte response factor was observed to be proportional to concentration over four orders of magnitude when the electrolyte concentration is below 10^{-3} M. Therefore, optimal ESI method performance relies on balancing the independent requirements for liquid chromatography operation and efficient ESI.

Conversely, Kebarle and coworkers first recognized that gas-phase reactions could have a significant effect on ESI response [25–27]. The effect of gas-phase proton transfer reactions on the mass spectral responses of solvents and analytes with know gas-phase PA has been investigated, showing that the analyte response was either suppressed or eliminated in solvent systems with higher gas-phase PA than the analyte [16,28]. Kamel et al. have observed the significance of gas-phase proton transfer reactions on the ESI of

tetracyclines [20]. Their data strongly suggests that mechanisms in addition to solution ionization are involved in the formation of ESI sample ions.

Cocaine (COC), the major alkaloid of *Erythroxylum coca*, is a potent brain stimulant and one of the most vigorously addictive drugs. In vivo, COC is rapidly metabolized to benzoylecgonine (BZE) and ecgonine methyl ester (EME). Traditionally, these analytes have been monitored by GC, GC/MS [29,30], and LC/UV [31,32]. Recently, electrospray methods have been examined for quantitative and qualitative analysis of COC and its metabolites in biological matrices [1,2,4]. Routine analysis of forensic samples for EME, BZE, and COC require highly sensitive, accurate, and rugged assays.

The purpose of this study was to investigate the effects of electrolytes, solution pH and organic eluent composition on the ESI mass spectra and responses for COC, EME, and BZE in the positive ion mode. Mobile-phase additives are often used to improve chromatographic separations, increase analyte solubility, enhance ESI performance, and heighten ESI response of analytes [33]. The volatile mobile-phase additives employed in this study are commonly used reagents for reversed-phase liquid chromatography (RPLC)/ESI/MS analyses. Although particular electrolyte: pH: solvent combinations may yield the greatest electrospray responses, they may not provide optimum chromatography conditions. Our goal was to develop the optimum mobile-phase system for the analysis of EME, BZE, and COC by ESI/MS/MS. A better understanding of the synergy between solvent components and the ionization of analytes should facilitate future development of highly sensitive, accurate, and rugged assays.

2. Experimental

2.1. Materials

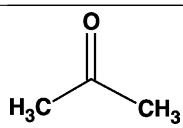
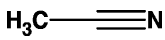
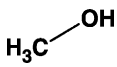
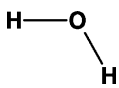
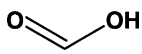
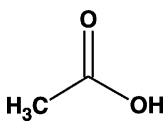
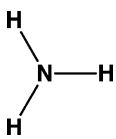
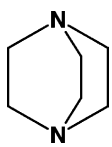
COC (free base), BZE hydrate and EME hydrochloride were obtained from Sigma (St. Louis, MO). A weighing of each analyte was made from neat material and standard stock solutions (5.0 mg/mL) of these compounds were prepared in HPLC grade acetonitrile obtained from J.T. Baker Company (Phillipsburgh, NJ) and stored at 4 °C. Dilutions of the standard stock solutions were made to provide a cocktail working stock solution (30 ng/μL) in HPLC grade acetonitrile. Analytical grade formic acid was obtained from Acros (Geel, Belgium), ammonium hydroxide from J.T. Baker Company (Phillipsburgh, NJ), and ammonium formate and ammonium acetate from Spectrum Chemical Mfg. Corporation (Gardenia, CA). We obtained HPLC grade water, acetone, acetonitrile, and methanol from J.T. Baker Company (Phillipsburgh, NJ). Solutions were filtered through a 0.45 μm TF (PTFE)® membrane filter, Gelman Sciences Inc. (Ann Arbor, MI).

2.2. Mass spectrometry and sample introduction

ESI/MS experiments listed in Table 1 were conducted using a Finnigan MAT Triple Stage Quadrupole (TSQ) 7000 mass spectrometer, with a Finnigan API source (San Jose, CA). The auxiliary gas and sheath gas pressures were set to 12 and 50 psi, respectively. ESI source voltage was 4.5 kV and the heated capillary was operated at 150 °C. Tube lens, capillary, axis offset, and lens 11 voltages were set to: 104.51, 37.71, −3.0, and −21.15 V. The data system consisted of a Compaq Pentium® III AP400 Professional Workstation (Houston, TX) operating Finnigan XCALIBUR Rev. 1.0 system software, Finnigan MAT (San Jose, CA). The instrument was operated in the positive ion mode, and 60 ESI spectra were collected over five periods by scanning the third quadrupole over a mass range of *m/z* 100–400.

To evaluate the effects of electrolytes, solution pH and organic eluent composition on the ESI mass spectra and responses for COC, EME, and BZE, samples were introduced into the ESI sources at an infusion rate of 5.0 μL/min, from a Hamilton Co. (Reno, NV) 250 μL gas-tight syringe, using a Harvard Model 11 syringe pump (South Natick, MA). The infusion syringe was connected to the electrospray sources via a 5.25 in. length of 0.0025 in. PEEK tubing. Pre- and

Table 1
Gas-phase basicities for mobile-phase eluents/additives, and substituted alkylamine at 25 °C

Component	Structures	Proton affinity (kcal/mol)
Acetone		196.7
Acetonitrile		188.2
Methanol		181.9
Water		166.5
Formic acid		178.8
Acetic acid		190.7
Ammonia		204
1,4-Diazabicyclo-[2,2,2]octane		228

post-flushing of the system for a period of 5 min with a 50:50 solution of water:methanol was performed to eliminate carryover between analyses. A 0.01 mM solution of reserpine in 50:50 HPLC grade water:methanol served as an external reference to monitor instrument drift over the course of the analyses.

2.3. Sample preparation

Ammonium formate, ammonium acetate, formic acid, and ammonium hydroxide were added to water to give solution concentrations of 5.0, 25, and 100 mM for each, and the pH of the individual solutions was measured. Equal (1:1) volumes of aqueous additive and 60% acetonitrile/40% acetone were thoroughly mixed, and the pH of the final mixture was obtained. This value is reported in Table 2. Data obtained for the two measurements did not vary by more than ±0.3 pH units. Before each infusion experiment 2.5 mL of the aqueous additive was added to 2.5 mL 60% acetonitrile/40% acetone, and thoroughly mixed. Then 167 μL of the 30 ng/μL cocktail standard in HPLC grade acetonitrile was added to give a final concentration of ∼1.0 ng/mL. A complete list of the mobile-phases investigated is given in Table 2.

3. Results and discussion

The structures of EME, BZE, and COC along with their molecular weights and acid dissociation constants (pK_a) are listed in Table 3 [34]. The pK_a values for EME and BZE were not available from the literature and were calculated using the Advanced

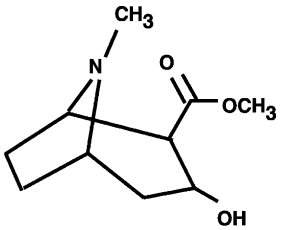
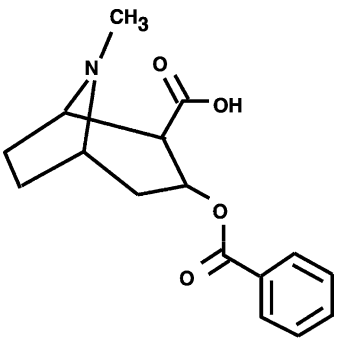
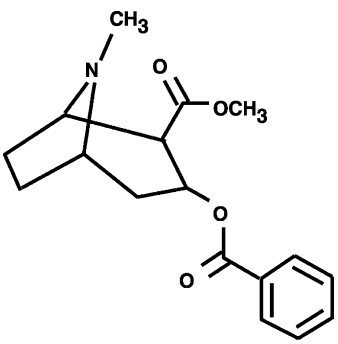
Table 2
Effects of mobile-phase additives on the positive ion ESI sensitivities of ecgonine methyl ester, benzoylecgonine, and cocaine

Analyte[a]	Mobile-phase additive	Apparent pH[b]	EME (199.2, 0.451)[c]	BZE (290.2, 0.311)[c]	COC (304.2, 0.297)[c]
$I[M + H]^+/C$	None	6.4	5.03	3.38	17.5
$I[M + Na]^+/C$	None	6.4		0.40	
$I[M + K]^+/C$	None	6.4		0.32	
$I[M + H]^+/C$	5.0 mM ammonium formate	5.8	6.12	8.91	22.4
$I[M + Na]^+/C$	5.0 mM ammonium formate	5.8		0.39	
$I[M + K]^+/C$	5.0 mM ammonium formate	5.8		0.27	
$I[M + H]^+/C$	25 mM ammonium formate	6.2	11.4	11.0	28.6
$I[M + Na]^+/C$	25 mM ammonium formate	6.2		0.13	
$I[M + K]^+/C$	25 mM ammonium formate	6.2		0.23	
$I[M + H]^+/C$	100 mM ammonium formate	6.4	12.3	13.2	33.7
$I[M + Na]^+/C$	100 mM ammonium formate	6.4		0.09	
$I[M + K]^+/C$	100 mM ammonium formate	6.4		0.20	
$I[M + H]^+/C$	5.0 mM ammonium acetate	6.6	3.57	4.73	13.6
$I[M + Na]^+/C$	5.0 mM ammonium acetate	6.6		4.73	
$I[M + K]^+/C$	5.0 mM ammonium acetate	6.6		4.73	
$I[M + H]^+/C$	25 mM ammonium acetate	6.8	3.41	2.95	13.0
$I[M + Na]^+/C$	25 mM ammonium acetate	6.8		2.95	
$I[M + K]^+/C$	25 mM ammonium acetate	6.8		2.95	
$I[M + H]^+/C$	100 mM ammonium acetate	7.0	4.61	3.50	17.0
$I[M + Na]^+/C$	100 mM ammonium acetate	7.0		3.50	
$I[M + K]^+/C$	100 mM ammonium acetate	7.0		3.50	
$I[M + H]^+/C$	5.0 mM formic acid	2.8	5.30	9.65	20.6
$I[M + Na]^+/C$	5.0 mM formic acid	2.8	5.30	0.10	
$I[M + K]^+/C$	5.0 mM formic acid	2.8	5.30	0.38	
$I[M + H]^+/C$	25 mM formic acid	2.7	4.68	10.6	17.9
$I[M + Na]^+/C$	25 mM formic acid	2.7		0.14	
$I[M + K]^+/C$	25 mM formic acid	2.7		0.18	
$I[M + H]^+/C$	100 mM formic acid	2.6	3.64	7.85	13.6
$I[M + Na]^+/C$	100 mM formic acid	2.6		0.11	
$I[M + K]^+/C$	100 mM formic acid	2.6		1.87	
$I[M + H]^+/C$	5.0 mM NH_4OH	9.2	4.26	4.89	19.2
$I[M + Na]^+/C$	5.0 mM NH_4OH	9.2			
$I[M + K]^+/C$	5.0 mM NH_4OH	9.2			
$I[M + H]^+/C$	25 mM NH_4OH	9.6	5.25	6.75	20.4
$I[M + Na]^+/C$	25 mM NH_4OH	9.6		0.32	
$I[M + K]^+/C$	25 mM NH_4OH	9.6		0.20	
$I[M + H]^+/C$	100 mM NH_4OH	10		7.04	17.4
$I[M + Na]^+/C$	100 mM NH_4OH	10		0.31	
$I[M + K]^+/C$	100 mM NH_4OH	10		0.31	

[a] Molar sensitivity expressed as $I[M + H]^+/C$, where I is the ion intensity (in arbitrary units) and C is concentration (mol/L).
[b] The pH of the additive in 60% acetonitrile/40% acetone (1:1, v/v) (represents the average of two readings from two different pH meters).
[c] MW and concentration (nM), respectively.

Chemistry Development Inc., ChemSketch pK_a Program [35]. Order of relative basicity for these compounds as indicated by the pK_a of the singly-protonated species are: EME > COC > BZE. Gas-phase PA for these compounds have not been determined. It maybe sufficient to estimate their PA's based on structural similarity with 1,4-diazabicyclo-[2,2,2]octane, which has a PA of 228 kcal/mol [36]. The gas-phase PA for the various modifiers and solvents used for this study are given in Table 1.

Table 3
Structures and ionization constants of ecgonine methyl ester, benzoylecgonine, and cocaine

Analyte[a]	MW	pK_{a_1}	pK_{a_2}
 **Ecgonine Methyl Ester**	199	9.3	14.2
 **Benzoylecgonine**	289	3.2	10.1
 **Cocaine**	303	8.61	N/A

[a] pK_a values were obtained from [34] and [35].

3.1. Positive ion electrospray mass spectrometry

The positive daughter-ion mass spectra for EME, BZE, and COC along with their proposed fragmentation pathways are shown in Fig. 1. Fig. 2 shows the detailed mechanistic decomposition of COC. Additionally, the decompostions for EME, BZE and COC have been previously investigated and reported elsewhere [4,37]. Fig. 3 depicts the positive ion mass spectra for EME, BZE and COC obtained utilizing different mobile-phase additives. Primarily $[M+H]^+$ ions were observed, although decomposition of EME and COC to ecgonidine methyl ester rendered a sizable peak at *m/z* 182.0. Adducts derived from addition of ammonium salts and ammonium hydroxide, along with cluster ions were not observed, although small amounts of cationized BZE as $[M + Na]^+$ and $[M + K]^+$ are visible in the spectrum.

Results summarizing the effects of various mobile-phase additives on the $[M + H]^+$ intensity of EME, BZE, and COC obtained with the Finnigan ion-source are given in Table 2. These data represent the average of ion intensities obtained from 60 spectra collected over five periods in rapid succession, from infusion of a 1.0 ng/mL cocktail solution of the analytes (0.297–0.451 nM). For comparison purposes the data is reported as $I[M + H]^+/C$, the protonated molecular ion intensity divided by the molar concentration of the analyte.

Mobile-phase additives and eluents that are frequently used for LC/ESI/MS analyses were examined buffers explored included 5.0, 25, and 100 mM solutions of ammonium formate, ammonium acetate, formic acid, and ammonium hydroxide. Ammonium formate and ammonium acetate were selected to investigate the effect of increasing volatile-buffer concentration on signal intensity, whereas formic acid and ammonium hydroxide were used to test the effect of varying pH on the ESI of the test compounds. There have been numerous reports in the literature describing the effects of organic modifiers on the positive and negative ion electrospray responses for various compounds [15,17–19]. However, we determined empirically that a 60:40 mixture of acetonitrile:acetone produced the most intense responses for the analytes using the Finnigan API source. In the 60% acetonitrile/40% acetone:H_2O (1:1) solvent system at pH 6.4, the molar sensitivity for COC is ∼5.0 times greater than BZE, and ∼3.5 times greater than EME. Data obtained under identical instrumental conditions, while employing MeOH as the organic modifier,

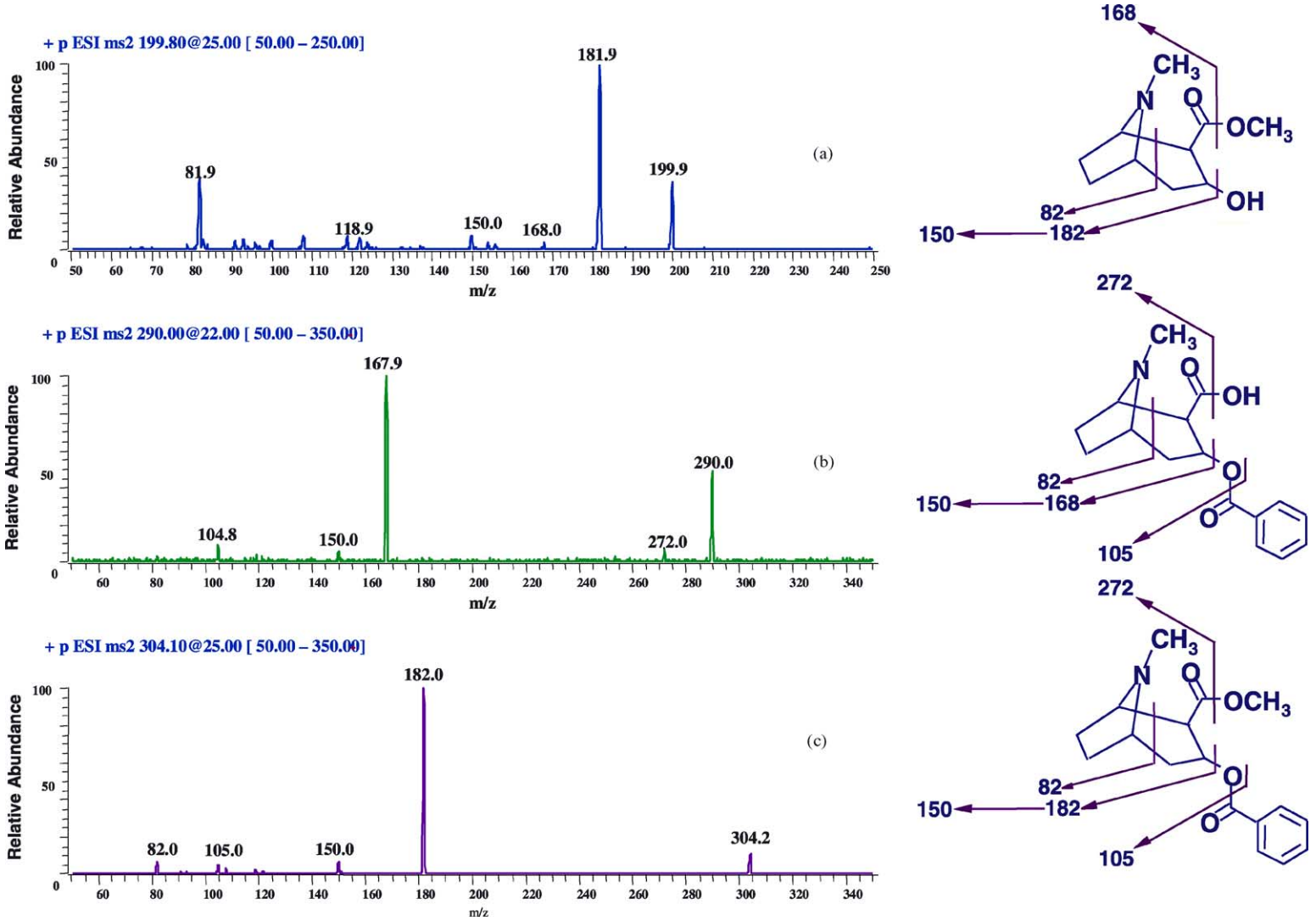


Fig. 1. Electrospray positive ion product mass spectra acquired for EME $[M + H]^+$ $m/z \rightarrow 200.1$ (a), BZE $[M + H]^+$ $m/z \rightarrow 290.2$ (b), and COC $[M + H]^+$ $m/z \rightarrow 304.2$ (c) at a collision cell gas pressure of 2.2 mTorr and collision energies of 22–25 eV, and their proposed dissociation pathways.

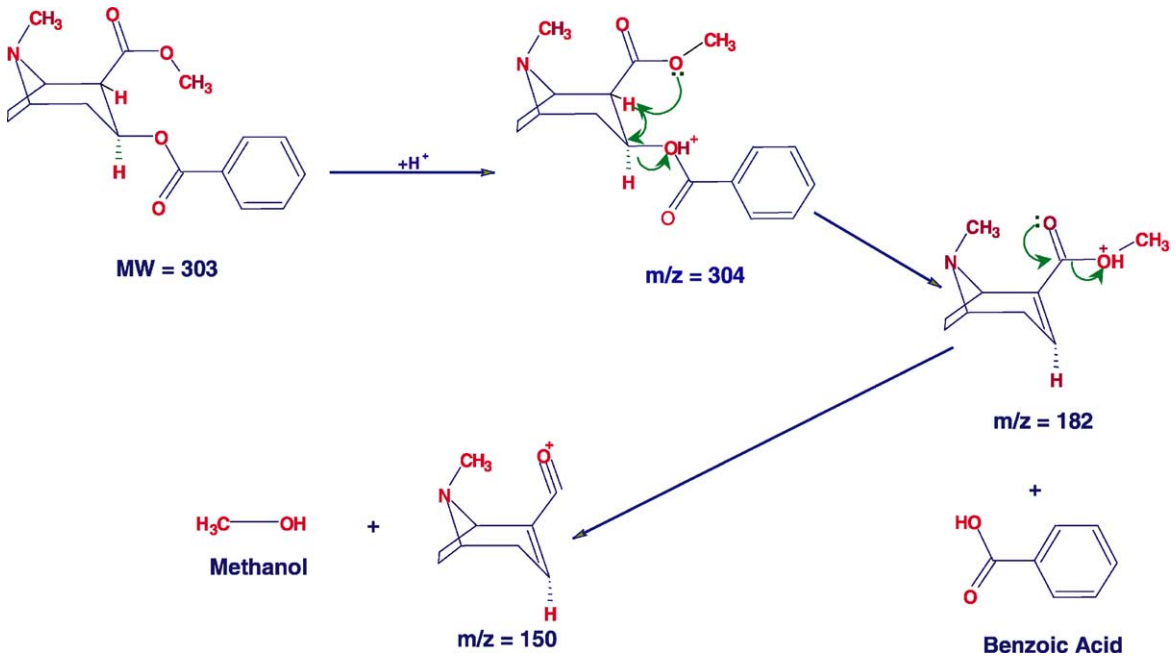


Fig. 2. Proposed mechanistic decomposition of COC to ecgonidine methyl ester and ecgonidine aldehyde at *m*/z 182.0 and 150.0, respectively.

shows a large decrease in molar sensitivities for EME and COC (∼50%). There was a negligible decrease observed for the molar sensitivity of BZE (∼7.4%).

The acid dissociation constants (pK_a) for EME, BZE, and COC are given in Table 1. Order of relative basicity for these compounds as indicated by the pK_{a_1} of the singly-protonated species is: EME > COC > BZE. At a specified pH, small differences in acid dissociation constants can have considerable impact on the portion of the analyte present as the protonated, deprotonated, and/or neutral species. Treating the analytes as monoprotic/diprotic acids, as indicated by their acid dissociation constants, the amount present as the protonated species can be calculated as a function of pH. Therefore, if pH is the sole factor in determining whether an analyte molecule protonates, then protonation will occur when pH < pK_a. When the pH > pK_a, protonation would not occur and the analyte would not be present in the mass spectrum. Following this corollary, one could rationalize that the higher the analyte pK_{a_1}, the lower the overall analyte sensitivity. In acidic solutions, pH ∼ 3.0, EME and COC exist primarily as the protonated species and the molar fraction present as $[M + H]^+$ is calculated to be approximately 0.99 for each. However, at a solution pH of 3.0, where the $pK_{a_1 BZE}$ = pH, the molar fraction present as $[M + H]^+$ is calculated to be only 0.50. The remaining BZE is observed as the neutral (in-solution) species. As the solution pH increases the fraction present as $[M + H]^+$ for the analytes decreases in the following order: BZE < COC < EME. For example, at pH 6.6, the amount of BZE present as $[M + H]^+$ is <0.1%, whereas greater than 99% of EME is present as $[M + H]^+$. Consequently, if molar sensitivity is a function dependent on the concentration of $[M + H]^+$ ions in solution, then EME should exhibit a higher response than BZE under the same analytical conditions. This generalization does have one possible caveat, the uncertainty of the droplet pH from which ions are produced. Under these conditions, it would suffice to say that one would expect the sensitivity obtained for EME to be greater than that for BZE, as EME is a more basic compound. To the contrary, our experiments found that at pH 6.6, BZE is

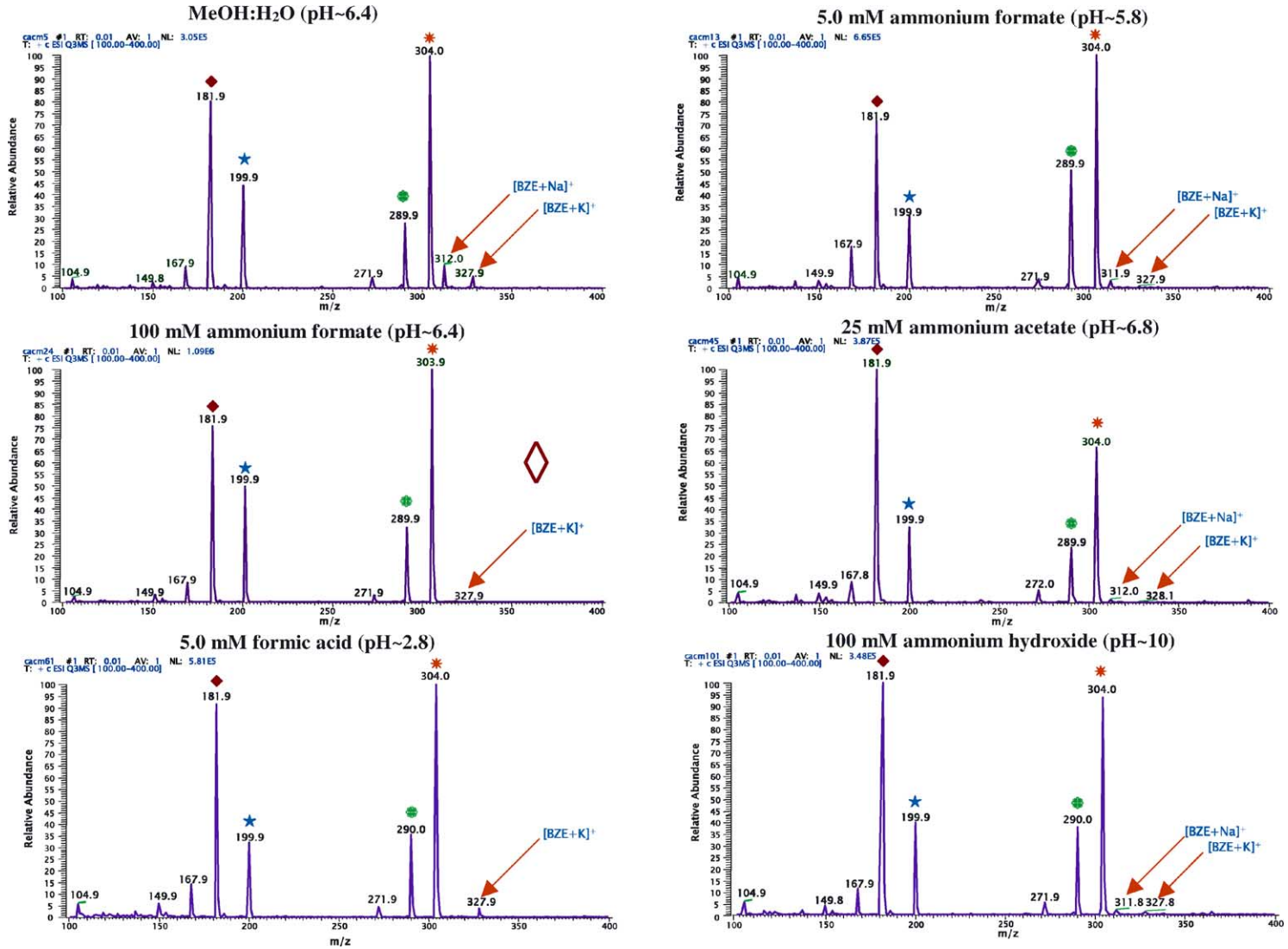


Fig. 3. Electrospray positive ion mass spectra of EME (MW = 199), BZE (MW = 289), and COC (MW = 303) with different mobile-phase additives. Asterisk denotes the highest intensity for $[M + H]^+$. The following symbols represent: (◆) decomposition of EME and COC to ecgonidine methyl ester, *m/z* 182; (★) $[EME + H]^+$; (●) $[BZE + H]^+$; (✱) $[COC + H]^+$. Samples (1.0 ng/μL) were infused at 5.0 μL/min, and 60 ESI spectra were collected over five periods by scanning the third quadrupole over a mass range of *m/z* 100–400.

~1.4 times the sensitivity of EME, although less than 0.1% of BZE is present as $[M + H]^+$ ions in solution.

For these analytes, when the $pK_{a\,BZE} < pH < pK_{a\,COC} < pK_{a\,EME}$ or $pK_{a\,BZE} < pK_{a\,COC} < pH < pK_{a\,EME}$, the sensitivities for BZE and COC would be suppressed, while enhancing the response for EME. However, at pH 2.6 the order of $I[M + H]^+/C$ for these compounds is COC > BZE > EME. When the solution pH is increased to ~9.2, this same order of $I[M + H]^+/C$ is observed, and the molar sensitivities for EME and COC are higher than those obtained at pH 2.6. At this pH, BZE and COC exist predominantly as the neutral species, while ~50% of BZE is found as $[M + H]^+$ ions. This divergence suggests that solution pH alone does not widely influence $I[M + H]^+/C$, and perhaps mechanisms other than extraction of ions from solution may be key in the formation of protonated molecular ions.

The addition of electrolyte to the test solutions provided mixed results when compared to the sensitivities attained with the MeOH:H_2O system. Generally, $I[M + H]^+/C$ for the test analytes decreased across the series: ammonium formate > formic acid > ammonium hydroxide > MeOH:H_2O > ammonium acetate. Addition of ammonium formate to the test solution resulted in increases of $I[M + H]^+/C_{avg}$ for EME, BZE, and COC by factors of 2.0, 3.3, and 1.6, respectively, when compared to the MeOH:H_2O system. Furthermore, the $I[M + H]^+/C_{avg}$ for EME, BZE, and COC observed with ammonium formate, are 31–50% greater than those achieved with ammonium acetate. Roughly, the $I[M + H]^+/C_{avg}$ that we observed with formic acid and ammonium hydroxide for EME, BZE, and COC were 15–54% and 33–53% lower, respectively, than those achieved with ammonium formate.

It has been reported that increasing the concentration of buffer additives leads to decreased analyte response in ESI [24,33]. We did not observe this effect in our studies of 5.0, 25, and 100 mM solutions of ammonium formate, ammonium acetate, formic acid, and ammonium hydroxide. Varying the electrolyte concentration of the test solutions provided mixed results when compared to the molar sensitivities attained with the MeOH:H_2O system. For example, the molar sensitivity obtained with 5 mM HCOOH, pH 2.8, is significantly larger for BZE (factor of ~3) as compared to that attained with the MeOH:H_2O system, while $I[M + H]^+/C_{EME}$ and $I[M + H]^+/C_{COC}$ remain relatively unchanged (5.4 and 15% increases, respectively). Across the ammonium formate series, a steady increase in $I[M + H]^+/C$ for the test analytes was observed with increasing ammonium formate concentration, from 5.0×10^{-3} M to 1.0×10^{-1} M. When using ammonium acetate, the $I[M + H]^+/C$ for EME and COC increase as the electrolyte concentration increases, however, BZE decreases. The molar sensitivity for BZE with 5.0 mM ammonium acetate at pH 6.6 is greater by a factor of ~1.4, as that achieved with 100 mM ammonium acetate at pH 7.0.

We observed a decline in the $I[M + H]^+/C$ of EME, and COC when varying the concentration of formic acid from 5.0×10^{-3} M to 1.0×10^{-1} M. This corresponds to a drop in molar sensitivities for these analytes of ~34% across the concentration range studied. However, BZE exhibited its highest $I[M + H]^+/C$ with 25 mM formic acid (pH 2.7), and the lowest with 100 mM formic acid (pH 2.6). The basis for the increase in the response of BZE is unclear, although it may be related to changes in the ionization status of BZE as the electrolyte concentration changes.

In contrast to the effects noted when employing formic acid, the use of ammonium hydroxide rendered distinct observations. As the ammonium hydroxide concentration is varied from 5.0×10^{-3} M to 1.0×10^{-1} M, the $I[M + H]^+/C$ for EME and COC initially increases by factors of ~1.2 and 1.0, respectively, then declines by factors of ~1.2 for each. A continual rise in $I[M + H]^+/C$ for BZE is observed across the concentration range investigated. At the pH's employed using the ammonium hydroxide solutions (9.2–10), 99% of BZE exists as the neutral species. For example, when using 100 mM ammonium hydroxide pH 10, the $I[M + H]^+/C_{BZE}$ is 34% larger than $I[M + H]^+/C_{EME}$, although a small fraction of EME is protonated at this solution pH. These results suggest that droplet surface charging effects may drive protonation of analytes when $pH > pK_a$.

Investigations have been conducted showing that gas-phase PA of solvent and analyte molecules can have a dramatic effect on spectra produced by ESI [14,16,28]. These studies have confirmed analyte response would be suppressed when the gas-phase proton affinity of a solvent species is higher than that of the analyte. In a sense, the solvent acts as a strong gas-phase base and extracts available protons from the analyte. The corollary is observed when solvents with weaker gas-phase PA are used, they are less capable of scavenging protons from the analyte, and analyte signal will be observed in the mass spectrum.

There have been reports on the effect of gas-phase proton transfer chemistry for compounds with $pK_a < 3.0$ [35]. These gas-phase processes are driven by the pK_a, gas-phase basicity of the analytes, and the composition of the electrolyte system. Although the gas-phase PA for EME, BZE, and COC have not been established, it maybe sufficient to estimate their PA's based on structural similarity with 1,4-diazabicyclo-[2,2,2]octane, which has a PA of 228 kcal/mol [36]. As noted by the gas-phase PA for the various modifiers and solvents used for this study in Table 1, ammonia has the highest gas-phase PA of 204 kcal/mol. Hence, if the PA's for the test analytes fall in the range of ~228 kcal/mol, then sufficient protons would be available for efficient proton transfer. Since our highest $I[M + H]^+/C$ for EME, BZE, and COC was obtained with 100 mM ammonium formate pH 6.4, this suggests that: (1) at this high electrolyte concentration, the PA's of the analytes are probably greater than those of the solvent species, (2) absence of ammonium adducts in the EME, BZE, and COC spectra, validate the estimation of PA's for these analytes, and (3) gas-phase processes in conjunction with solution chemistry may be important in generation of ions by ESI.

4. Conclusions

Mobile-phase eluents and additives had pronounced effects on the sensitivities of EME, BZE, and COC when analyzed by ESI in the positive ion mode. Generally, $I[M + H]^+/C$ for the test analytes decreased across the series: ammonium formate > formic acid > ammonium hydroxide > MeOH:H_2O > ammonium acetate. Of the solvent mixtures tested, 100 mM ammonium formate (pH 6.4) gave the greatest sensitivity for $[M + H]^+$ ions. There was no evidence of a correlation between pH and $I[M + H]^+/C$ for EME, BZE, and COC. Increasing the concentration of ammonium formate resulted in higher molar sensitivities for the test analytes, as compared to ammonium acetate. Cluster ions were not observed for any of the test species when employing ammonium salts and ammonium hydroxide. These data suggest that the PA's of the analytes are probably greater than those of the solvent species, and gas-phase processes in conjunction with solution chemistry may be important in generation of ions by ESI.

There are stringent constraints placed on eluent and modifier selection for use with ESI/MS. Since, many of these factors are poorly understood; a better understanding of the synergy between solvent components and the ionization of analytes should facilitate future development of highly sensitive, accurate, and rugged ESI/MS assays. We consider the work described in this article a first-step in developing a sensitive assay for the detection of EME, BZE, and COC in biological matrices.

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