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Comparison of Atmospheric Pressure Photoionization and Atmospheric Pressure Chemical Ionization for Normal-Phase LC/MS Chiral Analysis of Pharmaceuticals

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In this work, we compared APPI and APCI for normal-phase LC/MS chiral analysis of five pharmaceuticals. Performance was compared both by FIA and by on-column analysis using a ChiralPak AD-H column under optimized conditions. By comparison, APPI generated more reproducible signals and was less susceptible to ion suppression than APCI. APPI generated higher peak area and lower baseline noise, and therefore much higher S/N ratios. APPI sensitivity (i.e., S/N ratio) was approximately 2−130 times higher than APCI by FIA and was approximately 2.6−530 times higher than APCI by on-column analysis depending on specific compounds. The better APPI sensitivity as compared to APCI was more dramatic by on-column analysis than by FIA. APCI sensitivity was degraded by ion suppression caused by LC column bleeding components and by elevated APCI baseline noise relative to APPI. On-column APPI LODs (at S/N = 3) were 83, 16, 17, 95, and 7 pg for enantiomer #1, and 104, 23, 19, 122, and 17 pg for enantiomer #2 for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively, on a Waters ZQ. APPI offers no concern of explosion hazard relative to APCI corona needle discharge or ESI high voltage discharge when flammable solvents (e.g., hexane) are used as mobile phases. Whether APPI dopants are required depends on the IP(s) of mobile-phase solvent(s) and solvent complexes, and photon energies of VUV lamps. Dopant was not necessary for hexane-based mobile phases due to their self-doping effects. Dopants did enhance Kr lamp APPI sensitivity when MeOH was used as the mobile phase. However, dopants became unnecessary for the MeOH mobile phase when the Ar lamp was used.

Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are two atmospheric pressure ionization (API) techniques coupled with liquid chromatography/mass spectrometry (LC/MS), which are commonly used for normal-phase enantiomeric analysis of pharmaceuticals. Polysaccharide derivatives belong to the most widely used chiral stationary phases (CSPs) for enantioseparation. The most commonly used mobile phases in combination with the polysaccharide-based CSPs are mixtures of alkanes and alcohols (e.g., hexane/IPA, heptane/ethanol, etc.).[1,2] APCI and ESI are generally considered to be incompatible with normal-phase conditions when flammable mobile-phase solvents are used at high flow rate due to concern of potential explosion hazard caused by APCI corona needle discharge or ESI needle high voltage discharge. Two approaches have been applied to overcome this problem.

The first approach is to lower APCI probe temperature.[3,4] Ceccato and coauthors, for example, have developed a normal-phase APCI-LC/MS/MS method using a Chiralpak AD column for enantiomeric separation of tramadol and its active metabolite *O*-desmethyltramadol in human plasma with APCI vaporizer temperature set at 250 °C.[3] Because APCI ionization occurs in the gas phase, lower probe temperature may result in incomplete desolvation and vaporization of column effluents, therefore giving lower sensitivity.

The second approach is to add a large aqueous polar organic makeup flow by postcolumn addition to reduce the alkanes (e.g., hexane) concentration in the mobile phase prior to APCI or ESI source. This setup has been utilized by several researchers for normal-phase LC/MS chiral analyses of drugs when alkanes-based mobile phases were used.[5−12] One good example was normal-phase APCI-LC/MS/MS enantiomeric bioanalysis of several chiral drugs and their active metabolites in plasma including verapamil, norverapamil, oxybutynin, *N*-desethyloxybutynin, doxazosin, and

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sotalol.[5] The LC enantioseparations were performed on a 100 × 2.1 mm ID ChiralPak AD column under isocratic condition with a mobile-phase flow rate of 0.2 mL/min. A postcolumn reagent of IPA−0.025 mM ammonium acetate (75:25, v/v) was introduced at a flow rate of 0.8 mL/min to enable direct coupling of hexane-based mobile-phase chiral chromatography with the heated APCI nebulizer temperature set at 440 °C. A similar approach was used to minimize potential explosion hazard for normal-phase ESI-LC/MS enantioselective determination of terazosin in human plasma.[8] One disadvantage of this approach is the postcolumn dilutions of target analytes, resulting in elevated detection limits for trace quantitative bioanalysis.

Neat polar organic mobile phase (e.g., methanol or ethanol) offers enantioseparation for some drug analytes using polysaccharide-based CSPs. These mobile phases pose no concern of potential explosion hazard; however, APCI and ESI sample analyses using these mobile phases often require use of volatile mobile-phase modifiers (e.g., formic acid, ammonium formate, or ammonium trifluoroacetate) for sensitivity enhancement.[13,14] Use of alkanes-based mobile phase by APCI and ESI relies even more on mobile-phase additives for better detectivity and higher sensitivity.[5−12] This is especially true in the case of ESI. Yan et al. reported that, even with mobile-phase additives, TurboIonSpray (i.e., ESI) generated very low response for normal-phase enantiomeric analysis of MK-0767 in human plasma without the addition of an aqueous phase as the postcolumn reagent.[15] Not all chromatographic separations benefit from use of mobile-phase modifiers. The presence of additives in mobile phases going through LC columns may adversely affect the enantioseparation of analytes and, in some cases, shortens column life;[16] therefore, those additives solely used for improvement of sensitivity or ionization efficiency are often incorporated into the mobile-phase effluent flow by postcolumn addition prior to the ionization sources.

Atmospheric pressure photoionization (APPI) is an alternative ionization technique coupled with LC/MS for analysis of small molecules. APPI has been gaining in popularity since its introduction in the year 2000 mainly due to its ability to ionize compounds not ionizable by ESI and APCI. APPI has proved to ionize a wider range of polar and nonpolar drug and drug-like compounds with better ionization efficiency and higher sensitivity. Cai et al. compared the performance of APPI, APCI, and ESI using five sets of pharmaceutical standards and drug-like compounds.[17] APPI was found to give 100% detectable signals for the first set of 86 drug standards with diverse structures and polarities. More detailed studies were then performed to analyze another three sets of both drug standards and proprietary drug candidates. APPI again gave 100% detection probability with higher sensitivity than APCI and ESI for all 60 compounds. Most of the nonpolar compounds in these three sets were found not to be ionizable by APCI and ESI. Analysis of the final set of 201 Wyeth proprietary drug candidates gave overall 98% detectable signals by APPI versus 91% by APCI, and 91% by ESI, respectively. The authors concluded that APPI was a more universal ionization technique than APCI and ESI, offering great potential in high-throughput drug discovery especially for open access LC/MS applications.

APPI is less susceptible to matrix ion suppression and salt buffer effects than APCI,[18,19] and much less susceptible than ESI.[20,21] Furthermore, APPI and APCI offer comparable linear dynamic range (i.e., 4−5 decades) and much wider linear range than ESI.[20,22,23] APPI is more sensitive than APCI at low mobile-phase flow rate;[20] therefore, it is more suitable for trace quantitative bioanalysis with limited sample size using miniaturized LC column, making postcolumn addition of makeup flow unnecessary. APPI has also been identified as the ionization source, which exhibits the least dependency on use of mobile-phase additives for pharmaceutical screening.[17] APPI usually gives $[M]^+$, $[M + H]^+$, or $[M + H - xH_2O]^+$ as major ions with high sensitivity, avoiding the necessity of modifying mobile phases to monitor the adduct analyte ions such as $[M + NH_4]^+$. Unlike APCI and ESI, APPI offers no concern of potential explosion hazard, as APPI involves neither corona needle discharge nor ESI high voltage discharge. The APPI ionization process is initiated by photons emitted from VUV lamps (e.g., krypton lamp).[20] Also, APCI is prone to "carbon deposit" formation at the tip of corona needle and on the cone face, causing unstable signal and decreasing sensitivity over time. As a result, APPI has been identified and recognized in a recent journal review article as a preferred ionizer for normal-phase chiral analysis of pharmaceutical compounds.[24]

In this work, we compared the performance of APPI and APCI-LC/MS for normal-phase chiral analysis of five pharmaceutical drugs. A polysaccharide-based CSP, a ChiralPak AD-H column, in combination with both hexane-based mobile phase (i.e., hexane/isopropanol, hexane/ethanol) and polar organic mobile phase (i.e., 100% methanol) is utilized in this study.

EXPERIMENTAL SECTION

Chemicals and Reagents. Five pharmaceutical drugs were used for evaluation of APPI and APCI performance. They included benzoin (antiseptic agent, purity 99+%), naringenin (nutraceutical, purity ~95%), diperodon hydrochloride (anesthetic agent, purity >99%), mephenesin (muscle relaxant, purity 98%), and mianserin hydrochloride (antidepressant, purity >99%). These analytes were all purchased from Sigma-Aldrich, Inc., St. Louis, MO. The chemical structures of these compounds are presented in Figure 1. These compounds were selected because enantiomeric separations of these chemicals can all be achieved by the ChiralPak AD-H column (Chiral Technologies, Inc., West Chester, PA). Hexane, Chromasolv for HPLC, purity ≥95%, and ethanol (EtOH), HPLC grade, were both from Sigma-Aldrich, Inc. Methanol (MeOH), B&J ACS/HPLC certified solvent, was from Burdick &

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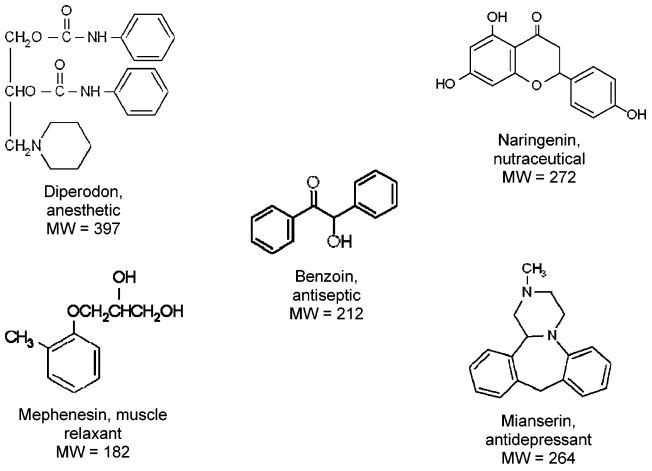

Figure 1. Chemical structure of analytes.

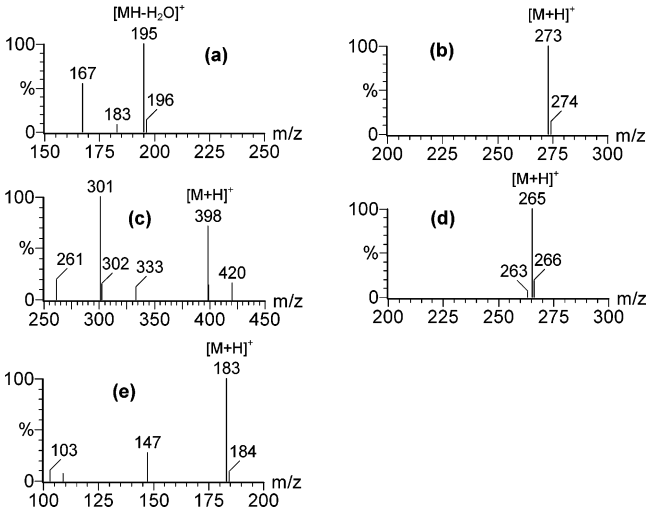

Figure 2. APPI+ full scan mass spectra. Injection amount = 50 ng (i.e., 10 ng/μL × 5 μL) each. (a) Benzoin, cone = 25 V; (b) naringenin, cone = 30 V; (c) diperodon, cone = 30 V; (d) mianserin, cone = 30 V; (e) mephenesin, cone = 10 V.

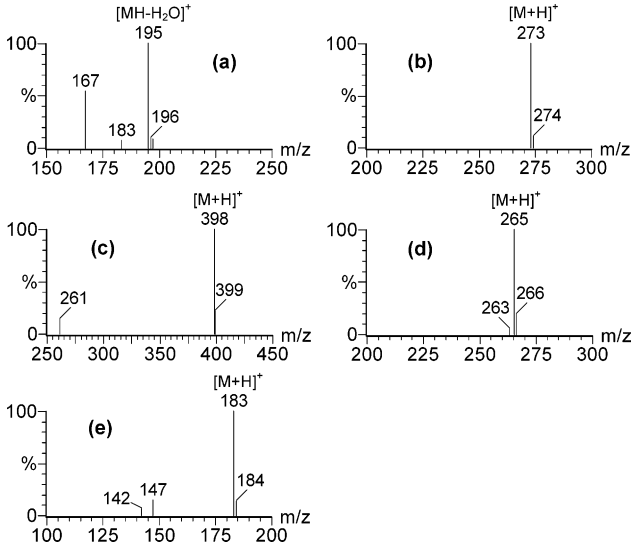

Figure 3. APCI+ full scan mass spectra. Injection amount and cone voltage are the same as those in Figure 2.

Jackson, Muskegon, MI. Isopropyl alcohol (IPA), Omnisolv, LC/GC grade, was from EM Science, Gibbstown, NJ.

Instrument, Parameters, and Conditions. A Waters Micromass ZQ LC/MS was used for these experiments. This instrument was equipped with an HPLC pump (Waters 2795 Separation Module), Syagen's PhotoMate APPI and APCI multimode source, and Masslynx v4.0 data acquisition and processing software. APPI and APCI parameters and conditions that were in common are as follows: extractor voltage, 3 V; RF lens voltage, 0.2 V; APCI probe temperature, 450 °C; desolvation gas flow rate, 400 L/h; acquisition dwell time, 0.2 s for flow injection analysis (FIA) and 1 s for on-column analysis. LC enantiomeric separation analyses were performed using a 150 mm × 2 mm, 5 μm ChiralPak AD-H column with mobile-phase solvents of 9:1 hexane/IPA for benzoin, 8:2 hexane/IPA for naringenin, 100% MeOH for diperodon, and 85:15 hexane/EtOH for mephenesin and mianserin at a flow rate of 0.2 mL/min under an isocratic condition. These pre-established mobile phases were adopted from the Applications CD (2006 Edition, Chiral Technologies, Inc.) The mobile phases used for FIA were the same as those used for on-column analyses. All APPI data were generated using a krypton (Kr) lamp unless specified. APPI repeller voltage was set at 0.7 kV. The optimized cone voltage and APCI corona needle discharge current were presented in the Results and Discussion.

Preparation of Standards. 1000 μg/mL (i.e., 1000 ppm) portions of benzoin, naringenin, and mianserin hydrogen chloride stock solutions were prepared in IPA. 1000 μg/mL portions of mephenesin and diperodon hydrochloride stock solutions were prepared in MeOH. Working solutions were prepared from these stock solutions by a series dilution method using their corresponding mobile phases as solvents.

RESULTS AND DISCUSSION

Comparison of APPI and APCI Mass Spectra. The full scan analysis of each analyte was performed using the above-described instrumental conditions and parameters. The data acquisitions were performed in both positive ion mode and negative ion mode simultaneously for each injection for each ionization source using Masslynx software. 50 ng (i.e., 5 μL of 10 ppm) of individual analyte standard was analyzed by FIA with a cone voltage ranging from 5 or 10 to 70 V in increments of 5 V. Positive ion mode was found to be much more sensitive than negative ion mode for the tested compounds for both APPI and APCI sources (generally ~2 orders of magnitude). Therefore, further analysis was focused on the positive ion mode only. Figures 2 and 3 show, respectively, APPI and APCI full scan mass spectra of target analytes at their optimum or near-optimum cone voltages. The effects of cone voltage on the major ion intensity are discussed in the next section. The results show that APPI and APCI give almost identical mass spectra for four out of five tested compounds. Benzoin gave $[M + H - H_2O]^+$, a protonated molecule with a neutral loss of H_2O (Figure 2a vs 3a). Naringenin, diperodon, mianserin, and mephenesin all generated protonated molecule $[M + H]^+$ as major ions with m/z 273 for naringenin (Figure 2b vs 3b), m/z 398 for diperodon (Figure 2c vs 3c), m/z 265 for mianserin (Figure 2d vs 3d), and m/z 183 for mephenesin (Figure 2e vs 3e). In addition to the common protonated molecule, m/z 398, produced by both APPI and APCI, diperodon also gave m/z 301 and m/z 420 as two other major ions by APPI. These two ions were not observed

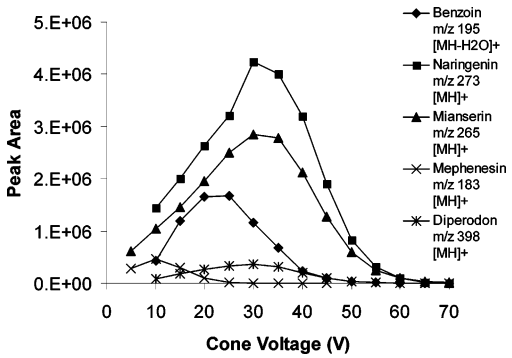

Figure 4. Effect of cone voltage on major ion peak intensity of analytes. 50 ng single point FIA. Optimum cone: benzoin = 20−25 V; naringenin, mianserin, and diperodon = 30 V; mephenesin = 10 V.

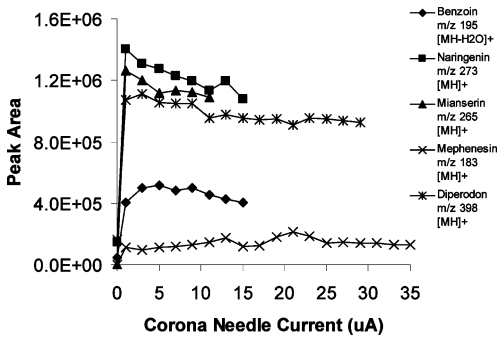

Figure 5. Effects of APCI corona needle discharge current (μA) on major ion intensity of analytes. 50 ng single point FIA. Optimum current: benzoin = 5 μA; naringenin and mianserin = 1−3 μA; mephenesin = 21 μA; diperodon = 3 μA.

in the APCI mass spectra. m/z 420 corresponds to sodiated molecule $[M + Na]^+$. We are not sure how the ion m/z 301 is formed. This ion appears to be the product ion of sodiated diperodon molecule with loss of one of the phenyl carbamate moiety (C_6H_5NHCO, see Figure 1) or the product ion of protonated diperodon molecule with loss of methyl piperidine fragment ($C_5H_{10}NCH_2$).

Optimization of Cone Voltage. The appearance of the mass spectra of a specific analyte is not only a function of ionization sources, mobile-phase solvents, and additives, but also a function of cone voltages. Cone voltage directly affects in-source fragmentation of target analyte and therefore the intensity of the major ions. In general, lower cone voltage tends to favor formation of protonated molecules and adduct molecules such as sodiated or ammoniated molecule adducts. Higher cone voltage favors formation of fragment ions. APPI and APCI full scans of each analyte were performed by FIA using the working mobile phases used for the separation of enantiomers of each specific analyte and with the conditions and parameters described above. 50 ng (i.e., 5 μL of 10 ppm) of analyte was analyzed per injection. Figure 4 shows the APPI signal intensities (i.e., peak area) of the extracted major ions as a function of cone voltages. As mentioned in the previous section, APPI and APCI gave very similar mass spectra for all tested compounds except for diperodon for which APPI generated two additional major ions of m/z 301, $[M - 96]^+$ and m/z 420, $[M + Na]^+$. The intensity of the fragment ion m/z 301 and sodiated molecule m/z 420 reached maximum at cone voltage of 35 and 20 V, respectively (these results were not shown in Figure 4 just for graphic simplicity). The maximum intensity of m/z 301 and m/z 420 was found to be approximately 1.58 and 0.59 times that of protonated molecule m/z 398 at their optimum cone voltages. Higher signal intensity does not necessarily mean higher sensitivity or higher S/N ratio. By comparison, the protonated molecule m/z 398 gave the highest S/N ratio among three major ions due to lower baseline noise. The effect of cone voltage on the intensity of a specific ion was found to be independent of ionization sources (i.e., APPI, APCI, or ESI) in agreement with our previous experiences. The cone effect by APCI analysis was performed for diperodon only and found to be consistent with the results obtained by APPI. Figure 4 shows that the optimum cone voltages for the selected major ions are 20−25 V for benzoin, 30 V for naringenin, mianserin, and diperodon, and 10 V for mephenesin. These cone voltages were used to generate S/N ratio for the target analytes for comparison of APPI and APCI performance.

Optimization of Corona Needle Discharge Current. The effects of APCI corona needle discharge electric current on mass spectra and ion intensity of target analytes were investigated by FIA using the parameters and conditions described above. The optimum discharge current is analyte specific and mobile-phase solvent dependent. The mobile phases and flow rate used for these FIA were the same as those used for on-column separations of enantiomers. 50 ng (i.e., 5 μL of 10 ppm) of individual analyte standard was injected per sample. APCI full scan analysis was performed at discharge currents of 0, 1, 3, 5, 7, and 9 μA, and proceeded at an increment of 2 μA per injection for the tested analyte until a clear trend of a plot (i.e., major ion intensity vs discharge current) was established as shown in Figure 5. It was observed that the magnitude of discharge current did not significantly affect the overall appearance of mass spectra or the relative intensity ratios of the ions on the mass spectra over the tested current ranges. However, the discharge current strength did affect the absolute intensity of the ions on the spectra. We find both from our previous experience and from the current tests that the discharge electric current range of 1−5 μA usually offers maximum ion intensities for many compounds. The optimum discharge current was found to be approximately 5 μA for benzoin, 1−3 μA for naringenin and mianserin, 21 μA for mephenesin, and 3 μA for diperodon.

Comparison of Peak Area. APPI and APCI peak area was compared by both FIA and on-column analysis using the optimized parameters and conditions described above. 50 ng (i.e., 5 μL × 10 ppm) of individual analyte was analyzed by FIA. 1, 10, and 100 ng (i.e., 10 μL of 0.1, 1, and 10 ppm) of individual analyte were, respectively, analyzed by on-column analysis. Three replicate analyses were performed at each concentration level in selected ion monitoring (SIM) mode by acquiring the major ions presented in Figure 4. Benzoin and mephenesin were found to be undetectable on-column by APCI for injections of 1 and 10 ng. Therefore, comparison of on-column peak area for these two compounds was based on 100 ng injection. The on-column peak area comparison for the other three compounds was based on 1 ng injection. Table 1 shows that APPI FIA peak area was, respectively, 2.3, 2.7, 2.3, 2.9, and 1.7 times higher than that of APCI for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively. The on-column APPI peak area (i.e., combined area of enantiomers #1

Table 1. Comparison of APPI and APCI Sensitivity by FIA and On-Column Analysis[a]

	LODs (pg) on-column APPI		LODs (pg) on-column APCI		S/N ratio on-column APPI vs APCI		peak area on-column APPI vs APCI			S/N ratio FIA APPI vs APCI	peak area FIA APPI vs APCI
name	#1	#2	#1	#2	#1	#2	#1	#2	#1 + #2	#1 + #2	#1 + #2
benzoin	83	104	44 379	54 397	535×	523×	3.4×	3.0×	3.2×	1.9×	2.3×
naringenin	16	23	133	204	8.3×	8.9×	2.7×	3.9×	3.2×	2.1×	2.7×
mianserin	17	19	41	51	2.4×	2.7×	3.2×	3.1×	3.2×	1.8×	2.3×
mephenesin	95	122	25 388	32 967	266×	271×	2.5×	2.5×	2.5×	132×	2.9×
diperodon	7	17	51	107	6.8×	6.4×	12.7×	12.1×	12.4×	2.0×	1.7×

[a] Average of triplicate analyses by SIM. Acquired ions are the same as those in Figure 4. #1 and #2 = enantiomer #1 and enantiomer #2. APPI diperodon data were generated using acetone as a dopant at 4 μL/min for FIA and 16 μL/min for on-column analysis. No dopants were used for the rest of the analytes. APCI on-column LODs for benzoin and mephenesin were determined for 100 ng injections due to low APCI sensitivity. All other on-column LODs were determined for 1 ng injections. FIA data were produced from 50 ng injections. Mobile phases used for FIA and for on-column separation were the same.

and #2) was, respectively, 3.2, 3.2, 3.2, 2.5, and 12.4 times higher than that of APCI for the same analytes in the same sequence. By comparing the APPI and APCI peak area difference between FIA and on-column analysis, we found that the on-column difference was generally more dramatic than that of FIA. This result is attributed to ion suppression caused by the ionization of LC column bleeding components. This observation further supports the conclusion made by previous researchers that APPI is less susceptible to ion suppression than APCI.[18,19,21] The ChiralPak AD-H column is packed with "coated" derivatized polysaccharide CSP containing amylose tris (3,5-dimethyl-phenyl carbamate). Coated phase columns are more susceptible to column bleeding than the columns packed with immobilized polysaccharide CSPs.[25] The ionization of column bleeding components not only caused ion suppression, resulting in lower analyte signal intensity, but also contributed significantly to the elevated APCI baseline noise as discussed in the following section.

Comparison of S/N Ratio. APPI and APCI peak-to-peak signal-to-noise (S/N) ratios were also compared by both FIA and on-column analysis using the above optimized parameters and conditions. The same analyses performed for peak area comparison described above were used to calculate the S/N ratio. The results are also presented in Table 1. The APPI FIA S/N ratio was 1.9, 2.1, 1.8, 132, and 2.0 times higher than that of APCI for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively. The improvement for APPI is even more dramatic for on-column analysis where we measured APPI S/N ratios that were 535, 8.3, 2.4, 266, and 6.8 times greater than for APCI (enantiomer #1) and 523, 8.9, 2.7, 271, and 6.4 times greater than for APCI (enantiomer #2) for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively. One extreme example was the case of benzoin. For this particular analyte, APPI FIA peak area was no more than 4-fold higher than that of APCI. However, on-column APPI S/N ratio was over 500-fold higher than that of APCI for both enantiomers (Table 1). Apparently, low APCI S/N ratio was mainly attributed to the elevated APCI baseline noise generated from APCI ionization of column bleeding components. The considerable on-column sensitivity difference of APPI and APCI relative to that of FIA was not what we first anticipated. Therefore, repeated experiments were performed, and very consistent results were obtained.

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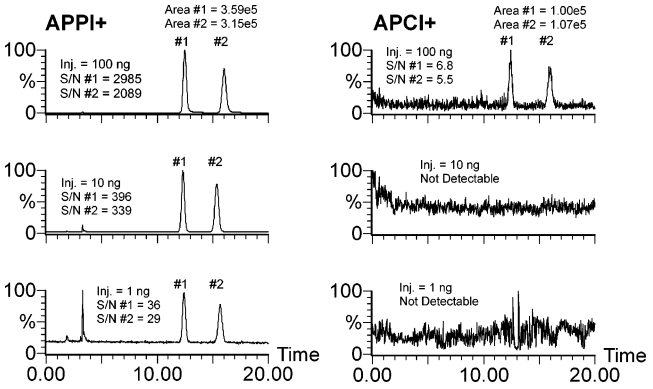

Figure 6. LC separation of benzoin enantiomers. SIM with *m*/*z* 195. APPI and APCI peak areas differ by no more than 4×, but S/N ratios differ by over 2 decades. Poor APCI sensitivity was mainly due to elevated baseline noise generated from ionization of column bleeding components. 150 × 2.1 mm, 5 μm, ChiralPak AD-H column. 9:1 hexane/IPA at 0.2 mL/min, isocratic. No dopant used. Injection of 10 μL of 0.1, 1, and 10 ng/μL standards, respectively.

Figure 6 shows enantiomeric separation of benzoin using the ChiralPak AD-H column for injections of 1, 10, and 100 ng, respectively. The ion chromatograms show that 1 and 10 ng of benzoin were not detectable on-column by APCI due to the combined effects of ion suppression and elevated APCI baseline noise with the later as a major contributor. APPI and APCI noise level difference was calculated by blowing up representative sections of baseline and comparing their intensity readings on ion chromatograms. It was estimated that on-column benzoin APCI baseline noise level was approximately 27−43 times higher than that of APPI (results not shown). Figures 7−10 show triplicate on-column enantiomeric separation of naringenin, diperodon, mianserin, and mephenesin, respectively. The chromatograms of naringenin, diperodon, and mianserin were based on 1 ng injection level. Because 1 and 10 ng mephenesin were not detectable by APCI on-column, ion chromatograms generated from 100 ng injection were presented for direct comparison. By looking at the triplicate analyses of ion chromatograms, peak area, and S/N ratios presented in these figures, it is obvious that APPI not only gave higher on-column sensitivity, but also generated more reproducible signals than APCI. We are not sure why APCI baselines were so much noisier than those of APPI. Two possible reasons are: (a) APCI ionization of column bleeding components contributed to elevated baseline levels, and (b) we visually

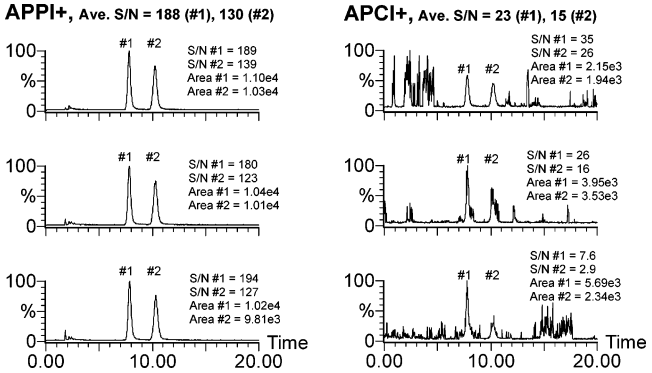

Figure 7. LC separation of naringenin enantiomers. SIM with *m*/*z* 273. Triplicate analyses showing sensitivity and signal stability of APPI and APCI. 150 × 2.1 mm, 5 μm, ChiralPak AD-H column. 8:2 hexane/IPA at 0.2 mL/min, isocratic. No dopant used. Injection amount = 1 ng (i.e., 0.1 ng/μL × 10 μL) each.

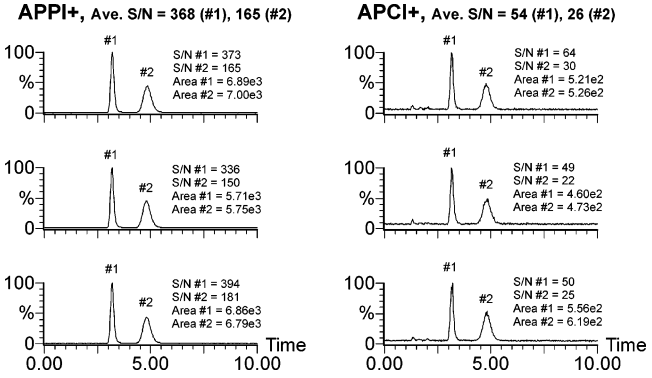

Figure 8. LC separation of diperodon enantiomers. SIM with *m*/*z* 398. Triplicate analyses showing sensitivity and reproducibility of APPI and APCI. 150 × 2.1 mm, 5 μm, ChiralPak AD-H column. 100% MeOH at 0.2 mL/min. APPI acetone dopant flow rate = 16 μL/min. Injection amount = 1 ng (i.e., 0.1 ng/μL × 10 μL) each.

observed uneven electron discharge (sparking) at the tip of corona needle, giving spuriously erratic baselines as shown in Figure 7.

Comparison of Limits of Detection. APPI and APCI on-column limits of detection (LODs) were determined from triplicate analyses of 1 ng (i.e., 10 μL of 0.1 ppm) injection of individual target analytes for all compounds with both ionization sources except for APCI for benzoin and mephenesin. These two compounds were not detectable on-column at 1 and 10 ng injection by APCI. Therefore, their APCI LODs were estimated from 100 ng injection level. The APPI LODs (at S/N = 3) were found to be 83, 16, 17, 95, and 7 pg for enantiomer #1, and 104, 23, 19, 122, and 17 pg for enantiomer #2 for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively. The APCI LODs (at S/N = 3) were found to be 44 379, 133, 41, 25 388, and 51 for enantiomer #1, and 54 397, 204, 51, 32 967, and 107 for enantiomer #2 for benzoin, naringenin, mianserin, mephenesin, and diperodon, respectively (Table 1). By comparison, the APPI LODs for benzoin and mephenesin were both over 2 orders of magnitude lower than those of APCI, making APPI a superior ionization source for normal-phase chiral analysis of these two compounds.

Effects of Mobile Phases, Dopants, and Photon Energies on APPI Sensitivity. Acetone and toluene were tested as APPI dopants for these target analytes using a Kr lamp. The effects of dopants on APPI sensitivity were found to be more dependent on the ionization potentials (IPs) or ionization energies (IEs) of

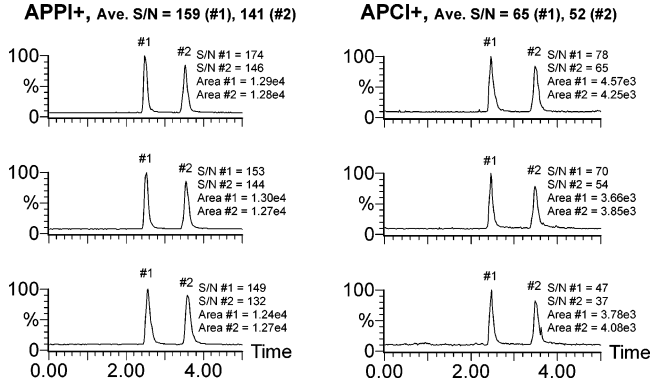

Figure 9. LC separation of mianserin enantiomers. SIM with *m*/*z* 265. Triplicate analyses showing sensitivity and reproducibility of APPI and APCI. 150 × 2.1 mm, 5 μm, ChiralPak AD-H column. 85:15 hexane/EtOH at 0.2 mL/min. No dopant used. Injection amount = 1 ng (i.e., 0.1 ng/μL × 10 μL) each.

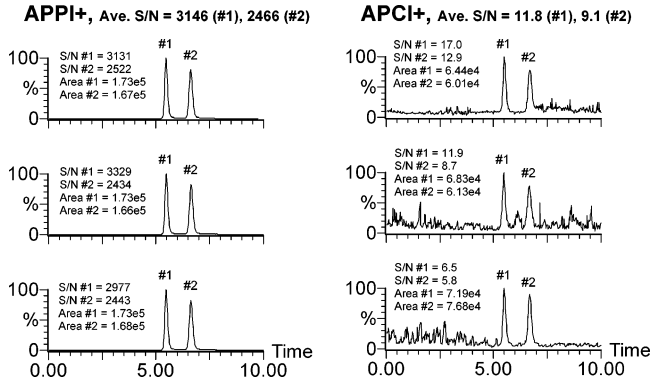

Figure 10. LC separation of mephenesin enantiomers. SIM with *m*/*z* 183. Triplicate analyses showing sensitivity and reproducibility of APPI and APCI. 150 × 2.1 mm, 5 μm, ChiralPak AD-H column. 85:15 hexane/EtOH at 0.2 mL/min. No dopant used. Injection amount = 100 ng (i.e., 10 ng/μL × 10 μL) each.

mobile-phase compositions rather than on the IPs or IEs of these target analytes. In other words, whether a dopant was needed was found to depend on how ionizable the mobile-phase solvents are. The more ionizable the solvents are, the less dependent it is on dopant. When hexane-based mobile phases (i.e., hexane/IPA and hexane/EtOH) were used, neither acetone nor toluene was found to enhance the APPI sensitivity of target analytes. Use of dopant was found unnecessary or non-critical due to self-doping effects of these two hexane-based mobile phases. This observation is consistent with what we observed for other analytes (e.g., lipids) using hexane or hexane/IPA as mobile phases (unpublished data). This is also in agreement with findings reported by others.[26] The IPs of hexane, EtOH, and IPA are, respectively, 10.13, 10.48, and 10.22 eV,[27] which are lower than Kr photon energies of 10.0 or 10.6 eV. Hexane gave an intense peak of *m*/*z* 85 or $[M - H]^+$. EtOH gave *m*/*z* 47, *m*/*z* 93, and *m*/*z* 139, and IPA gave *m*/*z* 61, *m*/*z* 121, and *m*/*z* 181, each corresponding to $[M + H]^+$, $[2M + H]^+$, and $[3M + H]^+$, respectively. The relative intensities of these ions were affected by cone voltage. Higher cone voltage favors formation of protonated molecule, and lower cone voltage favors formation of protonated dimers and trimers.

(26) Hsieh, Y. In *Using Mass Spectrometry for Drug Metabolism Studies*; Korfmacher, W., Ed.; CRC Press: Boca Raton, FL, 2004.
(27) NIST Webbook, http://webbook.nist.gov/chemistry.

MeOH differs from EtOH and IPA in that MeOH has an IP of 10.84 eV, which is higher than the photon energies of Kr lamp and therefore not ionizable directly. However, formation of MeOH dimers (IP = 9.74 eV)[28] in the gas phase lowers the IP of MeOH, making it ionizable indirectly. Similar to EtOH and IPA, MeOH also gave major ions of protonated molecules, protonated dimers, and trimers. However, protonated MeOH molecule is believed not to be formed from direct photoionization but from in-source fragmentation of protonated MeOH dimers and trimers. These product ions are produced by in-source collision-induced dissociations (CID).[29] Similar to EtOH and IPA, higher cone voltage favors formation of protonated MeOH molecule. In all of these cases, indirect APPI or APPI-initiated APCI plays a major role in achieving high APPI sensitivity. APPI photon energies ionize mobile-phase solvents, which react with target analytes, forming analyte ions. These ionized mobile-phase solvents served as dopants, therefore eliminating the necessity of using acetone, toluene, or other ionizable solvents as dopants or making use of dopants less critical. This process applies to hexane-based mobile phases or any other mobile phases, which are ionizable directly or indirectly. Nevertheless, some mobile phases and/or their dimers are more ionizable than others, which in turn affect the sensitivity of APPI. MeOH, in this case, is not as ionizable as hexane due to higher IP.

When MeOH itself was used as a mobile phase, both acetone and toluene were found to enhance the APPI sensitivity. Acetone was found to perform better than toluene as a dopant for diperodon. Whereas toluene enhanced both signal intensity and background noise of target analyte, acetone enhanced only the signal intensity of the target analyte and not the baseline noise. As a result, acetone gave a higher S/N ratio. Surprisingly, the background noise levels with acetone as a dopant were typically lower than those without using dopant. This phenomenon was observed in the case of not only diperodon analysis using MeOH as mobile phase, but also analyses of some other analytes (e.g., saturated and unsaturated triacylglycerol lipids) using many other mobile phases. These data will be published in a separate article.[30] In those cases, acetone was found to suppress the background noise levels of analytes. We are unsure of the mechanism for this effect; however, one possible reason is due to neutralization of background noise contributing ions. Acetone dopant enhanced diperodon peak area by a factor of only 2.3, but increased the S/N ratio by a factor of 8.4 when tested by FIA (Table 2). It was also interesting to note that the optimum acetone dopant flow rate (i.e., 4 μL/min) by FIA was different from that (i.e., 16 μL/min) by on-column analysis. Apparently, the optimum dopant flow rate is affected by the presence of column bleeding components. This implies that the optimum dopant flow rate will depend on sample conditions, such as the presence of matrix material from column or other sources.

We mention two other methods that give high APPI sensitivity for enantiomeric analysis of diperodon without the need for dopant. The first is to use pure EtOH as the mobile phase, which with the ChiralPak AD-H column offers excellent separation of diperodon enantiomers (result not shown). Because EtOH and its dimers in the gas phase are very ionizable by the photon energies of the Kr lamp, dopants are not necessary due to the self-doping effect of the solvent. A similar effect is observed for MeOH as the mobile phase when using an Ar lamp. The Ar lamp photon energy of 11.7 eV exceeds the IP of MeOH (i.e., 10.84 eV), making it very ionizable. By flow injection analysis without dopant, use of an Ar lamp enhanced diperodon peak intensity by a factor of 2.7, and increased S/N ratio by a factor of 6.4 as compared to use of a Kr lamp (Table 2). The greater enhancement of S/N as compared to peak intensity indicates that the Ar lamp gave lower baseline noise than the Kr lamp. The property of Ar lamp to generate higher signal intensity and lower baseline noise than Kr lamp was also observed when a few polyaromatic hydrocarbons (PAHs) were analyzed by APPI using MeOH as a mobile phase.[29] However, the advantage of using an Ar lamp must be weighed against its shorter life span as compared to a Kr lamp. The lifetime (to 50% brilliance) of a Kr lamp under typical LC/MS operation is about 2000 h; we have less lifetime information on an Ar lamp, but estimate it to be about 100−200 h under LC/MS conditions. These figures will vary from instrument to instrument.

Table 2. Effect of Dopant and Photon Energies on Diperodon Sensitivity[a]

APPI lamp	peak area	S/N ratio	peak area factor enhanced	S/N ratio factor enhanced
Kr, no dopant	$5.10 \times 10^{+05}$	$1.03 \times 10^{+04}$		
Kr, acetone dopant	$1.19 \times 10^{+06}$	$8.63 \times 10^{+04}$	2.3×	8.4×
Ar, no dopant	$1.37 \times 10^{+06}$	$6.60 \times 10^{+04}$	2.7×	6.4×

[a] Average of three replicate FIA by SIM with m/z 398.2. Mobile phase = MeOH at flow rate of 200 μL/min. Injection amount = 50 ng of diperodon hydrochloride. Dopant flow rate = 4 μL/min. MeOH IP = 10.84 eV. Kr lamp photon energies = 10.0 and 10.6 eV. Ar lamp photon energy = 11.7 eV.

The majority of chiral separations are accomplished using polysaccharide-based chiral stationary phases, and the most commonly used mobile phases are mixtures of alkanes and alcohols.[1] Hexane/IPA and hexane/EtOH were used in this comparison study. Other enantioseparation mobile phases were not tested. They include heptane/IPA, heptane/EtOH, hexane/THF, hexane/chloroform, hexane/dichloromethane, and acetonitrile.[2] Some of these mobile phases are limited to be used with immobilized polysaccharide-based CSPs. Based on the IPs of these solvents, we expect the following general trends: (a) dopants would enhance Kr lamp APPI sensitivity using mobile phases containing acetonitrile (IP = 12.20 eV), chloroform (IP = 11.37 eV), and dichloromethane (IP = 11.33 eV), and (b) high APPI sensitivity would be achieved without dopants if heptane/EtOH, heptane/IPA, and hexane/THF are used due to an expected self-doping behavior. In addition to IPs and photon energies, other parameters such as proton affinity and photoabsorption cross sections of mobile-phase solvents, dopants, and target analytes are also important factors affecting APPI sensitivity. More comprehensive studies of the effects of these parameters on APPI sensitivity were presented in separate articles.[29,30]

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CONCLUSION

APPI is the preferred ionizer for normal-phase chiral analysis because APPI (i) is more sensitive than APCI especially at low mobile-phase flow rate and therefore more suitable for quantitative trace bioanalysis with limited sample size using miniaturized LC column; (ii) is more specific and offers less ion suppression than APCI; (iii) generates much more reproducible signals than APCI especially at low mobile-phase flow rate; and (iv) offers no concern of explosion hazard relative to APCI corona needle discharge or ESI high voltage discharge when flammable solvents (e.g., hexane) are used as mobile phases; and also (v) APCI is prone to "carbon deposit" formation at the tip of corona needle and on the cone face, causing unstable signal and decreasing sensitivity over time.

Whether or not an APPI dopant is required greatly depends on the ionizability of the mobile-phase solvents or their dimers. Generally speaking, the more ionizable are the mobile phases, the more sensitive is the APPI, and the less dependent it is on use of dopants. Use of dopants was not necessary or not critical when hexane-based mobile phase was used. On the other hand, dopant enhanced APPI sensitivity for MeOH as a mobile phase when using a Kr lamp, but dopant was not necessary when using an Ar lamp due to the higher photon energy. Optimum dopant flow rate may also be sample matrix dependent.

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