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High-performance liquid chromatography-atmospheric pressure photoionization/tandem mass spectrometry for the detection of 17alpha-ethinylestradiol in hepatocytes

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ABSTRACT

Atmospheric pressure photoionization (APPI) as an interface for the high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) system was employed for the direct determination of 17alpha-ethinylestradiol (EE_2) in the incubation mixtures to support *in vitro* hepatic clearance studies. For the APPI source, the radical cation of the analyte via charge exchange with the dopant radical cation was used for the detection of EE_2 in the positive ion mode. It was demonstrated that the major signals of EE_2 in the acetonitrile/water mobile phase were substantially increased by replacing toluene with anisole as the dopant. The effects of several experimental conditions on the photoionization efficiency of EE_2 in the dopant-assisted APPI source were explored. Electrospray ionization (ESI) source was also suitable for the analysis of the analyte; however, ESI required a derivatization step prior to analysis. The applicability of the proposed HPLC–APPI–MS/MS approach following a protein precipitation procedure for the determination of EE_2 at low nano-mole levels was examined with respect to assay specificity and linearity. The assay results obtained by both HPLC–APPI–MS/MS and HPLC–ESI–MS/MS methods were in good agreement.

The swift growth in high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) applications is mainly due to the introduction of the atmospheric pressure ionization (API) interfaces between HPLC and a tandem mass spectrometer. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have dominated the ionization sources for the HPLC–MS/MS systems for qualitative and quantitative analysis of small molecules over the last decade [1–5]. ESI normally produces little fragmentation forming both protonated and de-protonated molecules for polar compounds in both positive and negative ion modes, respectively [4]. In contrast to ESI, APCI generates ions for less polar compounds [4]. Atmospheric pressure photoionization (APPI) is a relatively new ionization interface and has a potential as an alternative ionization source for HPLC–MS/MS for pharmaceutical analysis [6,7]. There is no universal ionization source for HPLC–MS/MS system to cover all target compounds. ESI will normally analyze around 80% of new chemical entities, necessitating a source change to analyze the remaining 10–20% of compounds [8]. In general, among the commercially available ionization sources, APPI and APCI perform best for non-polar compounds, while ESI performs best for polar compounds [9]. New instrumental developments have resulted in combined sources such as APCI/ESI and APPI/APCI for on-line HPLC–MS/MS [8,9]. These new combined sources have reduced the analysis time of sample plates by eliminating the need for a source hardware change, source optimization, and repeat analyses. Alternately, chemical derivatization is the other widespread and powerful approach for enhancing the ionization efficiencies of non-polar and low proton affinity (PA) compounds by HPLC–ESI/MS/MS analysis [10,11].

Previously, we demonstrated the potential of using APPI as a complimentary ionization technique with APCI and ESI and showed its applicability to the quantitative analyses of small molecules in biological fluids [12–15]. In this work, we compare the performance of ESI and APPI for the determination of a low proton affinity analyte, 17alpha-ethinylestradiol (EE_2), in an incubation mixture. The ionization efficiency of EE_2 through charge exchange with the addition of anisole for APPI was found to be approximately two orders of magnitude higher than that with toluene. The ion responses for the analyte cation, protonated solvent clusters, and dopant radical cation with APPI were monitored in order to explore the possible routes of ionization in positive ion APPI. The influences of several key factors such as ion transfer voltage, the dopant flow rate, and the composition of mobile phase on the ionization efficiency of the analyte under the reversed-phase HPLC conditions were also stud-

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ied. Matrix ionization suppression under experimental conditions for the proposed HPLC–ESI/MS/MS and HPLC–APPI/MS/MS methods was examined. The suitability of ESI and APPI techniques for the indirect and direct determination of EE_2, respectively, was further confirmed through correlation of the analytical results obtained by both ionization sources in terms of accuracy.

1. Experimental

1.1. Reagents and chemicals

EE_2 as the analyte was purchased from MP Biochemicals (Solon, OH, USA). Loperamide as the internal standard (ISTD) was purchased from Sequoia Research Product (Pangbourne, UK). Acetonitrile, toluene, and anisole were purchased from Fisher Scientific (Pittsburgh, PA, USA). Dansyl chloride (98%) and sodium bicarbonate (99.7%) were purchased from Acros Organics (New Jersey, USA). Deionized water was generated from a Milli-Q water purification system purchased from Millipore (Bedford, MA, USA) and in-house high-purity nitrogen (99.999%) was used.

1.2. Equipment

The HPLC system consisted of a Leap autosampler with a refrigerated sample compartment (set to 10 °C) from LEAP Technologies (Carrboro, NC), a Shimadzu on-line degasser, LC-10AD VP pump and LC-10A VP controller (Columbia, MD, USA). An Onyx Monolithic C18 (50 mm × 4.6 mm) from Phenomenex Inc. (Torrance, CA, USA) and a 50 mm × 2.1 mm HALO^{TM} C18 2.7 μm column (MAC-MOD Analytical, Inc., Chadds Ford, Pennsylvania, USA) were used as the analytical column for ESI and APPI, respectively. The mobile phases A and B composed of 100% water and 100% acetonitrile containing 0.1% formic acid, respectively, were used for reversed-phase chromatography.

Tandem mass spectrometric detection was performed using PE Sciex (Concord, Ontario, Canada) Model either API 4000 or 5000 triple quadrupole mass spectrometers equipped with heated nebulizer (APCI), turboionspray (ESI) or photospray (APPI) probes. The post-column infusion system was routinely employed as a standard measure for investigation of the matrix effect in our laboratory [16–19]. The APPI source was a second-generation PhotoSpray source from Applied Biosystems/MDS Sciex (Foster City, CA, USA), designed for their API 4000/5000 series of mass spectrometers.

1.3. Standard and sample preparation

Stock solutions of EE_2 and loperamide were prepared as 1 mg/mL solutions in methanol. Standard solutions were prepared by serial dilution in methanol/water. The *in vitro* hepatocyte incubation procedures used for predicting hepatic clearance were reported elsewhere [20]. Standard curves of 2, 1, 0.5, 0.25, and 0.125 μM were prepared under the same incubation conditions. For the HPLC–ESI/MS/MS method, a derivatization procedure reported elsewhere [21] was modified and applied prior to tandem mass spectrometric detection. Briefly, 20 μL standard or study incubation mixtures were pipetted into 96-well plate and the solvent were evaporated to dryness under a stream of nitrogen. 50 μL Sodium bicarbonate aqueous solution (100 mM, pH 11) was added to the residue, followed by vortexing for 2 min. Then 50 μL of dansyl chloride in acetone (1 mg/mL) was added. The 96-well plate was vortexed for 5 min and kept in a 60 °C water bath for 5 min to facilitate derivatization. A 10 μL sample was directly injected onto the HPLC–ESI/MSMS system for analysis.

1.4. Chromatographic conditions

The reversed-phase chromatographic separation was achieved using a two-solvent gradient system with mobile phases A and B. For the HPLC–ESI/MS/MS method, a gradient from 1% to 90% mobile phase B for separation of dansyl-EE_2 derivative and loperamide was run over 0.8 min, held for 0.3 min and re-equilibrated to 1% B over 0.4 min at a constant flow rate of 2.0 mL/min. The total run cycle time was less than 2.5 min. For the HPLC–APPI/MS/MS method, a ballistic gradient for elution of EE_2 and loperamide was used as follows: 0.2 min (25% B), 0.6 min (100% B), 0.9 min (100% B), 1.0 min (25% B) and finished at 1.1 min. The mobile-phase flow rate for HPLC was maintained at 1 mL/min. The retention times for EE_2 and the ISTD were 0.59 and 0.57 min, respectively. The effluent from the HPLC systems was connected directly to the ESI and APPI interfaces prior to the mass spectrometric detection without splitting.

1.5. Mass spectrometric conditions

The tandem mass spectrometers were operated in positive ion mode. The ESI conditions were as follows: source temperature, 550 °C; ion source voltage, 5000 V; declustering and entrance voltages, 60 and 10 V, respectively; ion source gas 1 and 2 setting, 50 and 20, respectively (arbitrary units); curtain gas setting, 25 (arbitrary units). The APPI conditions were as follows: source temperature, 450 °C; ion transfer voltage, 1000 V; declustering and entrance voltages, 60 and 10 V, respectively; ion source gas 1 and 2 setting, 30 and 20, respectively (arbitrary units); curtain gas setting, 20 (arbitrary units). The MS/MS reaction selected to monitor dansyl-EE_2 derivative and the ISTD was the transition from m/z 530 and m/z 477, to a product ion at m/z 171 and m/z 266 with the collision energy of 47 and 37 eV, respectively. The MS/MS reaction selected to monitor EE_2 was the transition from m/z 296, the radical molecular ion (M^+), to a product ion at m/z 213 with the collision energy of 25 eV. The protonated molecules of the dansyl-EE_2 derivative and the ISTD and the radical molecular ion of the analytes were fragmented by collision-activated dissociation with nitrogen as collision gas at a pressure of instrument setting 6. The APPI system was comprised of a probe inlet to accept APCI probe for the liquid sample being nebulized prior to inducing ionization, a power supply for the krypton lamp for photoionization, a nitrogen supply for cooling the lamp, a HPLC pump for dopant delivery, and a dopant inlet to introduce dopant to the nebulizer gas. Either toluene or anisole as a dopant was continuously introduced into the heated nebulizer at 100 μL/min for the determination of EE_2. The ion transfer voltage for photospray probe was set at 1.0 kV. Data were acquired and processed using Analyst 1.4.2 software (PE Sciex).

2. Results and discussion

2.1. Development of HPLC–ESI/MS/MS methods

HPLC–MS/MS systems combined with the protein precipitation technique for sample extraction are the standard procedure used to quantitatively determine drug components in our laboratory [22,23]. Although the ESI source covers a wider application range, the APCI source is frequently employed as an ionization interface for drug metabolism and pharmacokinetic (DMPK) screenings of new chemical entities. The chemical structure of EE_2 is shown in Fig. 1. Owing to the lack of nitrogen-containing functional groups such as amine, steroid-like compounds typically have poorer ionization efficiency for ESI than APCI as compared to other polar organic compounds. The use of ESI in both positive and negative modes was our initial attempts during the method development for the

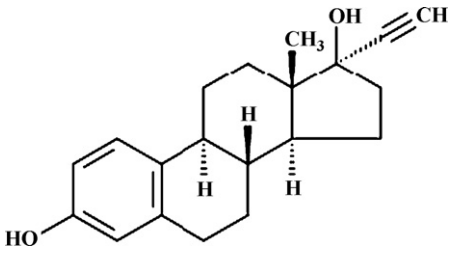

Fig. 1. Chemical structure of EE_2.

direct detection of EE_2. However, under the reversed-phase conditions with acetonitrile/water containing formic acid, EE_2 gave poor ionization efficiency in both positive and negative ion modes not sufficient to meet the required detection limits. APCI was expected to improve the sensitivity for EE_2; however, the sensitivity improvement by APCI over ESI for the EE_2 measurement was not substantial. These results were found to be in a good agreement with the literature reports [21,24].

The utilization of "ionizable" derivatives is one of the effective ways to enhance ESI–MS analysis of simple alcohols, and steroids [21,24–28]. In this work, we conducted chemical derivatization with dansyl chloride to develop a high-sensitivity HPLC–ESI/MS/MS method for the determination of EE_2 as described in the journal articles [21,26–28]. The resulting derivative is a tertiary amine and expected to yield a strong protonated molecule using ESI in the positive ion mode. A sensitivity improvement of approximately 1000-fold by ESI was achieved for EE_2-dansyl derivative over underivatized EE_2 (data not shown) to quantify low levels of EE_2 in study samples. The product ion spectrum of the molecular ions of dansyl-EE2 derivative (m/z 530) is similar to that reported elsewhere [21,24] which provided a major product ion (m/z 171). Fig. 2 shows the extracted HPLC–ESI/MS/MS chromatograms of EE_2-dansyl derivative and the ISTD from a spiked standard sample at 2.5 μM.

2.2. Development of HPLC–APPI/MS/MS methods

It was reported that for certain neutral compounds with low proton affinity such as idoxifene and its alcohol metabolites [29,30] APPI outperformed both APCI and ESI in terms of ionization sensitivity and validation statistics. To date, HPLC–APPI/MS/MS has been successfully employed for the determination of globotriaosylceramides [31], ergosterol [32], and pharmaceuticals [12,33,34]. In this work, we focus on APPI, as an alternative method of introducing samples with a low proton affinity compound, EE_2, to a tandem mass spectrometer. In the HPLC–MS/MS systems, the APPI source is similar to the APCI source which vaporizes mobile phase with a heated nebulizer (350–500 °C) to generate a dense cloud of gas-phase analytes with minimal thermal decomposition. A dopant, usually toluene, delivered from a HPLC pump is also vaporized in the nebulizer gas within the source chamber. Ionization uses a krypton discharge lamp to emit 10 eV photons to form dopant radical cations. The photoions, formed in large quantity from the dopant initiate a cascade of ion-molecule reaction with the analytes and solvent molecules leading to the production of MH^+ of a given analyte M (by proton transfer if the PA of the analyte is larger than the protonated solvent clusters and the PA of the solvent cluster is larger than that of the dopant radical cation), or M^+ (by charge exchange if ionization energy (IE) of the analyte is less than the combination energy of the dopant radical cation) in the positive ion mode [6,7]. The proposed ionization processes were summarized as follows:

$$[\text{Dopant}] + h\nu \rightarrow [\text{Dopant}]^+ + e^-$$

$$[\text{Dopant}]^+ + M \rightarrow M^+ + [\text{Dopant}] \quad (\text{charge exchange})$$

$$[\text{Dopant}]^+ + n[\text{Solvent}] \rightarrow [\text{Dopant-H}] + [(\text{Solvent})_n + H]^+$$

$$[(\text{Solvent})_n + H]^+ + M \rightarrow n[\text{Solvent}] + [M + H]^+ \quad (\text{protontransfer})$$

The full-scan APPI mass spectrum of EE_2 in the presence of acetonitrile/water and toluene as dopant is shown in Fig. 3 which features two intense peaks for both molecular ion, M^+ at m/z 296 and isotopic molecular ion $[M+1]^+$ at m/z 297 indicating the presence of 20 carbons. As for the significance of the two EE_2 peaks, the intense M^+ signal demonstrates the effectiveness of the charge exchange ionization pathway under the experimental conditions. It was reported that the ionization responses for the analytes with low IEs and low PA in acetonitrile can be further enhanced 100-fold by using anisole-assisted APPI, achieving the same level of sensitivity as that for analysis of high PA analytes [35]. As shown in Fig. 4,

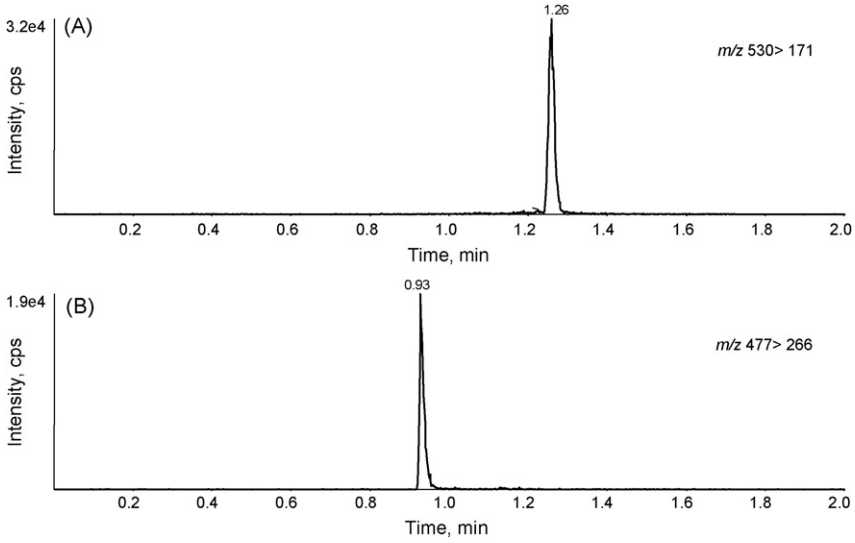

Fig. 2. The extracted HPLC–ESI/MS/MS chromatograms of (A) dansyl-EE_2 derivative and (B) loperamide from a standard sample at 0.125 μM.

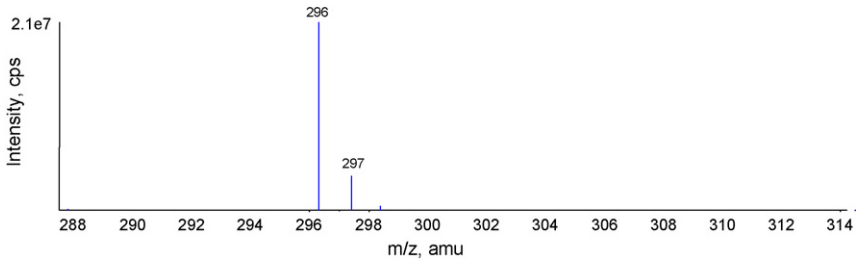

Fig. 3. APPI spectrum of EE_2 in the presence of acetonitrile/water and toluene as dopant.

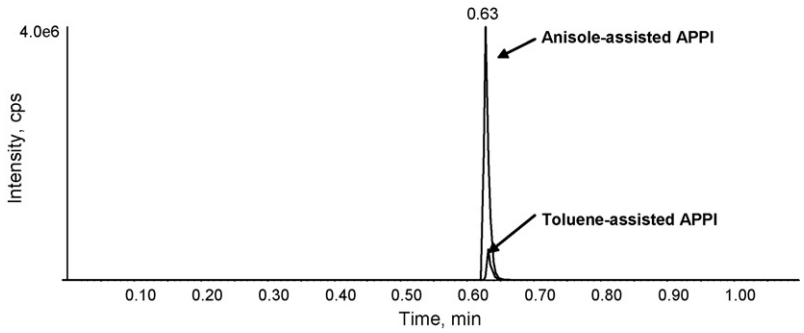

Fig. 4. The extracted HPLC–APPI/MS/MS chromatograms of EE_2 (M^+) using anisole and toluene as the dopant solutions delivered at 0.1 mL/min. The gradient HPLC conditions for the APPI interface are described in the text.

the use of anisole to replace toluene as the dopant promoted the formation of M^+ via charge transfer by a factor of at least 5 depending on the experimental conditions.

For the APPI–MS, source parameters such as temperature and ion transfer voltage have a strong impact on the assay detection limit [36]. Ion transfer voltage, an offset potential between the probe ion deflector and the mass spectrometer curtain plate, is responsible for transferring the analyte ions formed in the ionization region to the mass spectrometer. Fig. 5 shows the effects of the ion transfer voltages on the key M^+ signals at a constant mobile-phase flow rate of 1 mL/min in the presence of toluene. Fig. 5 indicates that a threshold voltage at 600 V was needed to move the ions toward the mass spectrometer. The ion signals increase as the ion transfer voltages increase up to 1000 V and then started to decrease.

The sensitivity for the analytes by APPI under the reversed-phase conditions was found to be a function of many factors such as solvent composition and flow rate [37,38]. In this work, we examine the effects of the solvent composition and the dopant flow rate on both the charge exchange and the proton transfer reactions in the dopant-assisted APPI system. Fig. 6(A) shows that the photoionization efficiency of EE_2 M^+ in the presence of toluene or anisole as dopant decreased as the proportions of acetonitrile in the mobile phase increased. In order to understand the fundamental ionization processes, the full-scan of dopant-assisted APPI spectra ranging from m/z 60 to m/z 200 were recorded in the presence of mobile phases A or B. Mobile phases A and B are water and acetonitrile containing 0.1% formic acid. The background spectra of the toluene and anisole changed substantially when mobile phases A or B were introduced to the APPI source. For toluene, the background ion intensities were reduced by 40% and 80% with the introduction of mobile phases A and B, respectively. The amount of other reactant ions and the protonated water/acetonitrile clusters such as $[CH_3CN + H_2O + H]^+$ (m/z 60) showed minimal change when water was replaced with acetonitrile as the solvent. However, the abundance of dopant radical cations of toluene, $C_7H_8^+$ (m/z 92) was found to be reduced by 50% when water was completely replaced with acetonitrile. The dopant radical cations of toluene

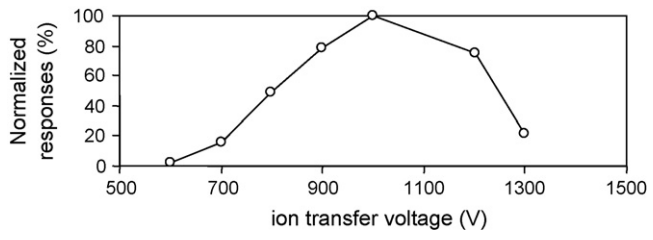

Fig. 5. Normalized APPI responses of M^+ of EE_2 as a function of ion transfer voltages.

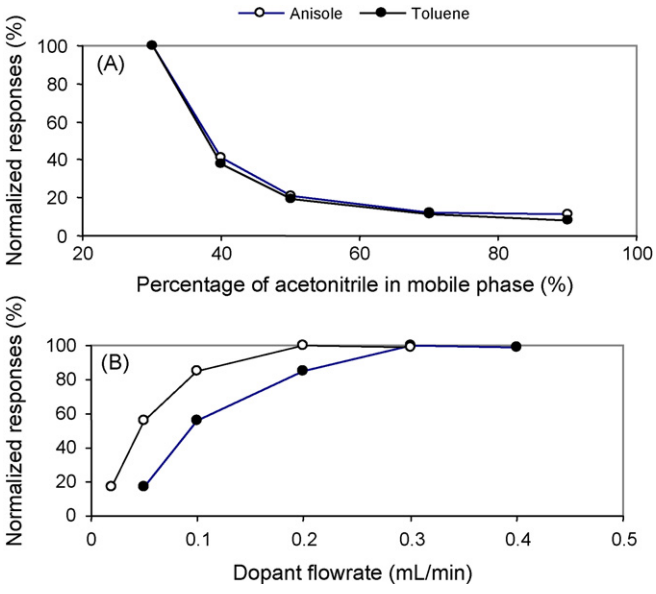

Fig. 6. Normalized APPI responses of M^+ of EE_2 as a function of (A) acetonitrile contents in the mobile phase and (B) dopant flow rate.

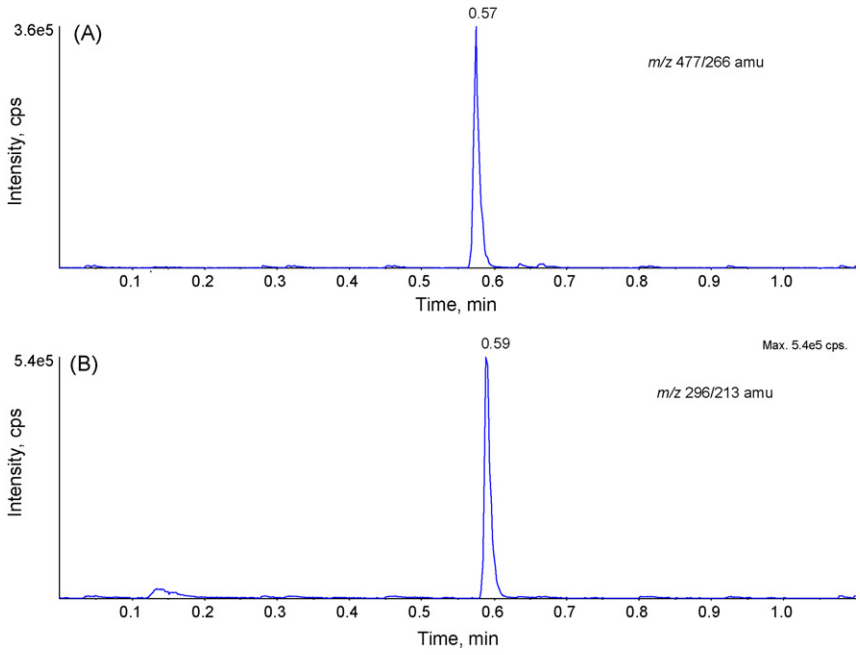

Fig. 7. The extracted HPLC–APPI/MS/MS chromatograms of (A) loperamide and (B) EE_2 from a standard sample at 0.125 μM.

were gradually depleted as the acetonitrile contents in the mobile phase increased. A decrease in the signal of analyte radical cations (M^+) at the higher ratios of acetonitrile in the mobile phase can be related to a decrease in the abundance of dopant radical cations. The decrease in APPI ionization efficiency for EE_2 at higher contents of acetonitrile in the mobile phase is explained likely a result of the depletion of toluene radical cations. A similar phenomenon in terms of APPI responses of EE_2 was also observed when anisole was employed as the dopant. Here, the background intensity was also found to be decreasing when water in the mobile phase was gradually replaced with acetonitrile. Thus, the above results suggest that the APPI efficiency of EE_2 is largely dependent upon the availability of the dopant ions in the gas phase.

Fig. 6(B) shows the relationship between dopant flow rate and the EE_2 M^+ signal. The plots for toluene and anisole in Fig. 6(B) show the steep increases in the analyte M^+ signal as the dopant flow in the HPLC–APPI/MS/MS system is initiated. Increasing dopant flow in the HPLC–APPI/MS/MS system at a constant mobile-phase flow rate will enhance the dopant-to-solvent ratio in the ionization source which in turn increases the density of the dopant photo-ions and the intensity of ion signals of the analytes. As expected, Fig. 6(B) shows that the EE_2 M^+ signals obtained by the APPI source increase with increasing the dopant flow rates up to 0.2 and 0.3 mL/min for anisole and toluene, respectively. However, a plateau in $M^{\bullet+}$ intensity appeared as the dopant solvents were driven over 0.2 and 0.3 mL/min for anisole and toluene, respectively. The primary dopant ion generation rate, R_P (ions s^{-1}) was suggested to be expressed as the product of the rate of photon absorption by the dopant and the total quantum yield of ionization [37,38]. At a constant mobile-phase flow rate, an increase in R_P was expected as the density of dopant is increased at high flow. For anisole and toluene over a dopant flow rate of 0.2 and 0.3 mL/min, respectively, further increases in R_P did not raise the analyte ionization efficiency. This phenomenon might be due to the collisional quenching of excited-state precursors to the dopant radical ions at high dopant flow leading toward a reduction of R_P. In addition, the plots in Fig. 6(B) did not overlap indicating that the kinetics of reaching plateaus in the formation of the analyte M^+ by APPI with increases in toluene or anisole flow was affected by the rate constants of charge exchange reaction between the analyte and the individual dopant radical cations.

The ionization suppression problem due to the matrix effect is a common factor to consider about assay reliability when developing a new HPLC–MS/MS method. The continuous post-column infusion experiments are the routine method for monitoring the ionization suppression for the HPLC–MS/MS approaches developed in this laboratory [16]. Any changes in the APPI responses of the infused EE_2 were assumed to be due to matrix ionization suppression caused by the sample-related materials eluting from the analytical column. The retention times of EE_2 and the ISTD appeared in the chromatographic region without matrix effect. Fig. 7 shows the extracted HPLC–APPI/MS/MS chromatograms of EE_2 and the ISTD using anisole as dopant from a study sample.

2.3. Comparison of the HPLC–MS/MS methods

No interfering peaks were found throughout the study samples indicating good specificity of the HPLC–ESI/MS/MS method for the

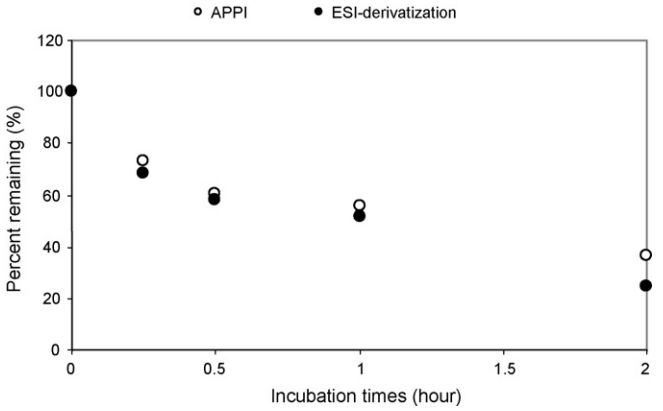

Fig. 8. Depletion of EE_2 from the hepatocyte incubation mixtures as a function of incubation times using HPLC–ESI/MS/MS with derivatization and direct HPLC–APPI/MS/MS methods.

measurement of dansyl-EE_2 derivative and the HPLC–APPI/MS/MS method for the measurement of EE_2. Calibration curves were linear with a good correlation over the working range by both ESI and APPI. The standard and study samples were independently analyzed for EE_2. The concentrations of EE_2 at different incubation time points determined by ESI or APPI methods are plotted in Fig. 8, respectively. The student *t*-test results indicated no significant difference within 95% confidence among these values for EE_2 obtained by the HPLC–APPI/MS/MS methods ($\alpha = 0.5$) with either toluene or anisole as dopant. The above results suggested that all of the direct or indirect HPLC–MS/MS methods for the determination of EE_2 in *in vitro* samples offered acceptable assay accuracy. Although the high-sensitive measurement of the analyte was not required for this *in vitro* study, Fig. 4 indicates that for the LC–APPI–MS/MS method the lower limit of quantitation for EE_2 is achievable at 0.001 μM.

3. Conclusions

HPLC–ESI/MS/MS in combination with a chemical derivatization with dansyl chloride was a widespread and sensitive approach for the determination of the trace amount of a low proton affinity compound, EE_2. In this work, a HPLC–APPI/MS/MS assay was developed for the direct determination of EE_2 in the course of *in vitro* metabolism studies. The APPI M^+ signals in the positive ion mode via charge exchange reaction with the dopant radical cations appeared to be the major molecular ions for the analytes that have low ionization energies and low proton affinities. The eluent composition, dopant flow rate and the selection of dopant solvents have a significant impact on the ionization efficiency of EE_2. The data indicated that the APPI efficiency of the charge exchange ionization pathway can be greatly enhanced by use of a carefully selected dopant at an appropriate flow rate. The results presented indicate that APPI can be an excellent complimentary tool to ionize compounds that are not readily ionizable by either ESI or APCI. The use of the APPI source for HPLC–MS/MS makes method development easier for the determination of EE_2 because no extra step for chemical derivatization is needed.

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References

[1] Y. Hsieh, W.A. Korfmacher, Curr. Drug Metab. 7 (2006) 479.
[2] Y. Hsieh, E. Fukuda, J. Wingate, W.A. Korfmacher, Comb. Chem. High Throughput Screen 9 (2006) 3.
[3] W.A. Korfmacher, Drug Discov. Today 10 (2005) 1357.
[4] W.M.A. Niessen (Ed.), The Encyclopedia of Mass Spectrometry, vol. 8, Elsevier Ltd., 2006.
[5] W.A. Korfmacher (Ed.), Using Mass Spectrometry for Drug Metabolism Studies, CRC Press, 2005.
[6] D.B. Robb, T.R. Covey, A.P. Bruins, Anal. Chem. 72 (2000) 3653.
[7] T.J. Kauppila, T. Kuuranne, E.C. Meurer, M.N. Eberlin, T. Kotiaho, R. Kostiainen, Anal. Chem. 74 (2002) 5470.
[8] R.T. Gallagher, M.P. Balogh, P. Davey, M.R. Jackson, I. Sinclair, L.J. Southern, Anal. Chem. 75 (2003) 973.
[9] L.C. Short, K.A. Hanold, S.S. Cai, J.A. Syage, Rapid Commun. Mass Spectrom. 21 (2007) 1561.
[10] L. Xu, D.C. Spink, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 855 (2007) 159.
[11] A. Ghanem, P. Bados, L. Kerhoas, J. Dubroca, J. Einhorn, Anal. Chem. 79 (2007) 3794.
[12] Y. Hsieh, K. Merkle, G. Wang, Rapid Commun. Mass Spectrom. 17 (2003) 1775.
[13] G. Wang, Y. Hsieh, W.A. Korfmacher, Anal. Chem. 77 (2005) 541.
[14] Y. Hsieh, C.J. Duncan, S. Lee, M. Liu, J. Pharm. Biomed. Anal. 44 (2007) 492.
[15] Y. Hsieh, G. Wang, K. Merkle, J.M. Brisson, W. Korfmacher, Anal. Chem. 75 (2003) 3122.
[16] Y. Hsieh, M. Chintala, H. Mei, J. Agans, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 15 (2001) 2481.
[17] J. Chen, Y. Hsieh, J. Cook, R. Morrison, W.A. Korfmacher, Anal. Chem. 78 (2006) 1212.
[18] Y. Hsieh, C.J. Duncan, J.M. Brisson, Anal. Chem. 79 (2007) 5668.
[19] Y. Hsieh, F. Li, C.J. Duncan, Anal. Chem. 79 (2007) 3856.
[20] Y.Y. Lau, E. Sapidou, X. Cui, R.E. White, K.C. Cheng, Drug Metab. Dispos. 30 (2002) 1446.
[21] F. Zhang, M.J. Bartels, J.C. Brodeur, E.L. McClymont, K.B. Woodburn, Rapid Commun. Mass Spectrom. 18 (2004) 2739.
[22] Y. Hsieh, C.J. Duncan, Rapid Commun. Mass Spectrom. 21 (2007) 573.
[23] Y. Hsieh, C.J. Duncan, J.M. Brisson, Rapid Commun. Mass Spectrom. 21 (2007) 629.
[24] W.Z. Shou, X. Jiang, W. Naidong, Biomed. Chromatogr. 18 (2004) 414.
[25] W. Li, Y.H. Li, A.C. Li, S. Zhou, W. Naidong, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 825 (2005) 223.
[26] M.R. Anari, R. Bakhtiar, B. Zhu, S. Huskey, R.B. Franklin, D.C. Evans, Anal. Chem. 74 (2002) 4136.
[27] G.J. Van Berkel, J.M. Quirke, R.A. Tigani, A.S. Dilley, T.R. Covey, Anal. Chem. 70 (1998) 1544.
[28] M.S. Rashed, L.Y. Al-Ahaidib, O.Y. Al-Dirbashi, M. Al Amoudi, M.M. Al-Sayed, Z. Rahbeeni, Z. Al-Hassnan, A. Al-Dbaas, M. Al-Owain, M. Ni Luanaigh, Anal. Biochem. 339 (2005) 310.
[29] J.A. Syage, J. Am. Soc. Mass Spectrom. 15 (2004) 1521.
[30] C. Yang, J. Henion, J. Chromatogr. A 970 (2002) 155.
[31] A. Delobel, S. Roy, D. Touboul, K. Gaudin, D.P. Germain, A. Baillet, F. Brion, P. Prognon, P. Chaminade, O. Laprevote, J. Mass Spectrom. 41 (2006) 50.
[32] M. Varga, T. Bartók, A. Mesterházy, J. Chromatogr. A 1103 (2006) 278.
[33] H.B. Theron, M.J. van der Merwe, K.J. Swart, J.H. van der Westhuizen, Rapid Commun. Mass Spectrom. 21 (2007) 1680.
[34] Y. Cai, D. Kingery, O. McConnell, A.C. Bach, Rapid Commun. Mass Spectrom. 19 (2005) 1717.
[35] T.J. Kauppila, R. Kostiainen, A.P. Bruins, Rapid Commun. Mass Spectrom. 18 (2004) 808.
[36] T.J. Kauppila, A.P. Bruins, R. Kostiainen, J. Am. Soc. Mass Spectrom. 16 (2005) 1399.
[37] D.B. Robb, M.W. Blades, J. Am. Soc. Mass Spectrom. 17 (2006) 130.
[38] D.B. Robb, M.W. Blades, J. Am. Soc. Mass Spectrom. 16 (2005) 1275.