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Atmospheric pressure photoionization liquid chromatographic–mass spectrometric determination of idoxifene and its metabolites in human plasma

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Abstract

This paper describes a comparison between atmospheric pressure chemical ionization (APCI) and the recently introduced atmospheric pressure photoionization (APPI) interface for the LC–MS determination of idoxifene and its major metabolite, SB245419 (SB19), in human plasma. The results indicate that analyte response in APPI is highly dependent on the solvent composition, especially to water in the mobile phase. Other parameters investigated are the mobile phase flow-rate, the chemical noise, and signal suppression by matrix interferences. APPI appears to be six to eight times more sensitive than APCI for idoxifene and its SB245419 metabolite; the response for the SB245420 metabolite is considerably better than for APCI conditions, but still not sufficient for trace level pharmacokinetic determinations in human plasma. The LOQ for the parent drug and its major metabolite were 10 and 25 ng/ml, respectively, in human plasma. From post-column infusion experiments we conclude that there is little difference in matrix suppression between APCI and APPI. From these studies we suggest APPI may be an additional tool in pharmaceutical LC–MS applications.

Keywords: Atmospheric pressure photoionization; Ionization methods; Idoxifene; SB245419

1. Introduction

Atmospheric pressure ionization (API) techniques have contributed greatly to the development and proliferation of liquid chromatography–mass spectrometry (LC–MS) techniques over the last 30 years [1–11]. Currently the two most common API techniques include electrospray and atmospheric pressure chemical ionization (APCI). The desired requirements for a useful liquid chromatographic system detector include high sensitivity and high selectivity [12], good response to a wide range of compound types, and ability to handle complex mixtures in a relatively high-throughput manner [13]. It is also helpful to have access to qualitative information [14] that is afforded by mass spectrometry as well as routine, rigorous quantitative analysis capability which has become so important in the pharmaceutical industry [15].

The benefits afforded by the two most common API techniques noted above meet the above criteria to varying degrees. For example, pneumatically assisted electrospray (ion spray) [11] is widely employed today, but occasionally suffers from relatively poor sensitivity due in part to the nature of the analyte(s) under investigation. Fortunately APCI

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techniques can sometimes provide complimentary coverage of analytes not well addressed with electrospray and by employing careful attention to the chemistry conditions APCI LC–MS techniques can provide very good LC–MS performance [16]. In a particular example from our laboratory we have for some time tried to improve the detection limits of estrogenic steroids. In a recent report [17] we elected to employ APCI liquid chromatography–tandem mass spectrometry (LC–MS–MS) techniques for the high-throughput trace detection of neutral steroidal compounds in human urine. Even after considerable effort to improve the detection limits for this work, the positive ion mode of APCI selected reaction monitoring (SRM) LC–MS provided a lower limit of quantification (LOQ) which was only in the low nanogram per milliliter range. In some applications there is an urgent need for at least a 1000-fold improvement in the LOQ for these compounds. Thus we need to continually seek improved detection limits from the analytical tools that we have.

Recently Bruins et al. [18] reported a new atmospheric pressure ionization (API) technique which they called atmospheric pressure photoionization (APPI). They proposed this ionization interface for liquid chromatography–mass spectrometry (LC–MS) could offer additional analytical capabilities for modern analytical problem solving and in some cases significant improvement in the detection limits of the technique depending upon what the target compounds are. Their report describes the photoionization process including the use of toluene as a dopant, wherein neutral compounds were reported to be more readily ionized than other approaches such as atmospheric pressure chemical ionization (APCI) and turboionspray. In addition, under optimized conditions, the photoionization approach reportedly provides APPI LC–MS higher detectability towards a wider range of compounds when compared with APCI LC–MS. In a thorough report on the effect of eluent on the ionization efficiency of flavonoids by ion spray, APCI, and APPI, Kostianen et al. [19] present a detailed study on the effects of solvent chemistry upon the performance of the title API techniques. Recently Van Berkel and Kertesz [20] described surface-assisted reduction of aniline oligomers, *N*-phenyl-1,4-phenylenediimine and thionin in APCI and APPI. This and the work of Kostianen et al. [19] employ relatively low HPLC flow-rates and highlight some of the strengths and potential pitfalls of the APPI technique. The work presented herein seeks to further characterize the analytical potential of this new APPI LC–MS interface while focusing on two drug metabolites which have been shown to display disappointing sensitivity using current API strategies.

We have selected the drug, idoxifene, whose quantitative LC–MS–MS determination has been reported previously [21–23]. Earlier studies in our laboratory have shown that under ion spray LC–MS conditions the pyrrolidinone metabolite (SB19) is at least six times less sensitive than the parent drug while the neutral primary alcohol metabolite, SB245420, is at least 100-fold less sensitive than the parent drug, idoxifine [23]. In fact, until recently we have considered the latter metabolite ‘‘transparent’’ to both ion spray and APCI LC–MS conditions; e.g. it could not be measured in biological samples due to our poor detection limits for this compound. The focus of this work was to compare APPI LC–MS and APCI LC–MS in terms of detection sensitivity, matrix effects, and experimental factors which affect the performance of the APPI technique.

2. Experimental

2.1. Chemicals

Idoxifene, d_5-idoxifene, and idoxifene metabolites SB245419 (SB19) and SB245420 (SB20) were kindly donated by SmithKline Beecham. HPLC-grade acetonitrile, hexane, and methanol were purchased from J.T. Baker (Phillipsburg, PA, USA). Isoamyl alcohol was obtained from Aldrich (Milwaukee, WI, USA). Deionized water was generated in-house with a Barnstead Nanopure II filtration system (Boston, MA, USA). Ammonium acetate and acetic acid were obtained from Sigma (St. Louis, MO, USA). Human plasma was purchased from Lampire Biological Laboratories (Coopersburg, PA, USA).

2.2. APPI and APCI source

A standard heated pneumatic nebulizer atmos-

pheric pressure chemical ionization source probe (Fig. 1A) from AB MDS SCIEX (Concord, Canada) was used in the comparison study. The APPI source was kindly provided by the research group of Dr Andries Bruins at the University of Groningen (Groningen, The Netherlands). The described system is comprised of a dopant delivery pump, a power supply for the photoionization lamp, and nitrogen nebulizing ''window'' gas for the lamp (Fig. 1B). The APPI interface was constructed by modifying a PE SCIEX Heated Nebulizer (HN) APCI source (Fig. 1A) to contain the nebulizer and the photoionization lamp and chamber. Discharge of the krypton photoionization lamp provides a continuous output of 10.0 eV photons. The deflection potential applied to the exit nozzle of the probe (Fig. 1B) was set at +1.2 kV relative to the ground. The dopant (toluene) was delivered at 50 μl/min with an ABI solvent delivery system (Applied Biosystems, Foster City, CA, USA). The heated nebulizer was maintained at 425 °C.

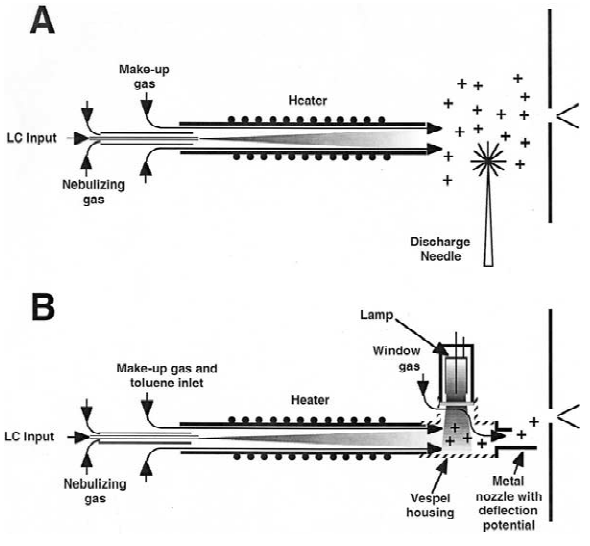


Fig. 1. Schematic representations APCI (A) and APPI (B) LC–MS interfaces. (A) Conventional heated pneumatic nebulizer LC–MS interface for APCI applications. Note that ionization is initiated via the corona discharge needle placed in the vaporized plume of mobile phase and volatilized analytes. In (B) a schematic representation of the described APPI interface is shown. This device is a modification of the probe shown in A and incorporates a krypton lamp affixed to the end of the heated nebulizer probe. In this device ionization is initiated by photoionization by the krypton lamp.

The heated nebulizer was also maintained at 425 °C.

2.3. Mass spectrometer

An AB MDS SCIEX API 365 triple-quadrupole mass spectrometer was used for all the experiments reported in this report. Sample Control (version 1.4) software was used to carry out selected-ion monitoring (SIM) LC–MS, full-scan LC–MS, and selected-reaction monitoring (SRM) experiments. All of the aforementioned experiments were carried out in positive ion mode. LC–MS acquisition files for LC–MS and LC–MS–MS analysis for each of the three analytes were tuned and optimized by LC2 Tune 1.4 instrument control and data acquisition software via direct infusion of sample post-column in the flowing stream of HPLC mobile phase. Unit mass resolution in Q1 and Q3 was set at 0.5–0.6 Da mass peak width at half-height, and nitrogen was used as the collision gas for CID experiments. Optimized tune files were used in LC–MS and LC–MS–MS analyses for the mixture of the three analytes. When the three analytes are separated via HPLC, the mass spectrometric analysis may be programmed by the Sample Control software to detect each analyte with its own optimized state file. A contact closure was established for communication between the mass spectrometer and the auto sampler for sample injection and LC separation.

2.4. Liquid chromatograph

All of the LC separations were performed at room temperature and in the isocratic mode with a Shimadzu LC-10AS pump. Sample injections were carried out by an SIL-10A/SCL-10A Shimadzu autosampler (Shimadzu Scientific Instruments, Columbia, MD, USA). Injection volume was set at 10 μl via injection loop. The LC column was 2 mm i.d×100 mm Betasil C_{18}, packed with 5 μm particles (Keystone Scientific, Bellefonte, PA, USA). Post-column infusion experiments were conducted by infusing the sample solution with a Harvard Syringe pump (Harvard Apparatus, South Natick, MA, USA) at a flow-rate of 5 μl/min and was introduced post-column into the HPLC effluent through an Upchurch Tee. Mobile phases with three composi-

tions were used and are described further below and varied from 0.1 to 1.0 ml/min.

2.5. SRM LC–MS matrix suppression experiments

A sample solution which contained 10 μg/ml of SB19 was infused post-column by a syringe pump at a flow-rate of 5 μl/min. The flow-rate of the LC mobile phase was set at 0.1 ml/min. while the data acquisition in the Sample Control was set as Manual Sync. The dwell time for the SRM APPI LC–MS determination of SB19 (m/z 538→112) was set at 300 ms. With the post-column infusion of the SB19 sample solution and the flow of the LC mobile phase established, data acquisition via SRM APPI LC–MS commenced. After establishing a 2-min stable ion current base line, the autosampler was manually triggered to inject 10 μl of either blank mobile phase or blank liquid–liquid extract of a human control plasma. Data acquisition was continued for another 10 min to allow sufficient time for the elution of the matrix components.

2.6. Sample preparation of human plasma for SRM LC–MS quantitative determination

Idoxifene and its major metabolite, SB19, were spiked into human plasma forming a series of standards containing 10, 50, 75, 250, 400, 500 ng/ml for idoxifene and 25, 50, 75, 250, 400, 500 ng/ml for SB19, and QCs at four levels (QC at LOQ: 25 ng/ml; low level QC: 60 ng/ml; medium level QC: 200 ng/ml; high level QC: 350 ng/ml each of idoxifene and SB19 in human plasma). For liquid–liquid extraction of the plasma samples, 100 μl of plasma sample was combined with 25 μl of internal standard (1000 ng/ml MeOH), and was extracted with 400 μl of 4% isoamyl alcohol in hexane [21]. Extraction efficiency for this method was determined to be within the range of 45–70%, depending on the QC levels [22]. The extracted organic upper layer was evaporated to dryness [24] and the dried residues were reconstituted in 100 μl of mobile phase and analyzed by SRM APPI LC–MS. Safety concerns in handling the human plasma were addressed similarly as previously reported [22].

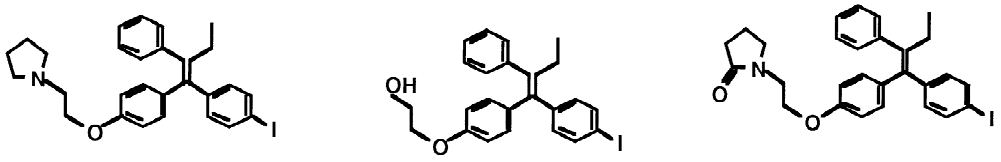
3. Results and discussion

3.1. Full-scan LC–MS determination of idoxifene and its two metabolites

APCI and APPI LC–MS full-scan analyses were performed on the three analytes as individual sample components. The chemical structures and their corresponding molecular masses are shown in Fig. 2. For idoxifene and its major metabolite pyrrolidone metabolite (SB19) shown in Fig. 2, the APPI LC–MS full-scan mass spectra are identical to those obtained from APCI LC–MS experiments (data not shown). The APCI LC–MS full-scan mass spectrum of the metabolite SB20 could not be obtained due to the lack of detection sensitivity in the APCI mode. APPI LC–MS determination of SB20 was obtained when a higher sample concentration of SB20 (100 times the concentration of idoxifene or SB19) was injected.

3.2. Effect of mobile phase composition on detection sensitivity with APPI and APCI

With APPI LC–MS, the LC effluent is vaporized



Idoxifene MW=523 SB20 MW=470 SB19 MW=537

Fig. 2. Structures of idoxifene, SB245420 (SB20), and SB245419 (SB19).

and subjected to photoionization before it is introduced to the mass spectrometer vacuum system. A number of steps are involved in photoionization [25]. These steps, in addition to photoionization, include photoexcitation, photodissociation, fluorescence, collisional quenching, recombination, and charge exchange. The photoionization efficiency of the analytes is collectively affected by these factors, and is directly related to the ionization potential (IP) of the molecules that are present in the ionization region. Ideally, the photons emitted by the lamp will transmit energy that can photoionize only the analyte molecules. In the preferred instance neither the solvent molecules in the mobile phase nor background matrix components would be ionized. In these experiments, the krypton discharge lamp emits 10.0 eV photons, which is insufficient to ionize the solvent molecules (water, IP=12.6 eV; methanol, IP=10.8 eV; acetonitrile, IP=12.2 eV). A dopant (toluene) was used in all APPI experiments and introduced to the ionization chamber [18] to facilitate photoionization of the targeted analytes. Toluene and other selected compounds have been discussed by Bruins et al. [18] as an aid to enhance the sensitivity for targeted compounds subjected to photoionization.

During the initial phases of this work it was discovered that the detection sensitivity of APPI LC–MS is affected by the composition of the LC mobile phases. In these studies 10 μl of a mixture containing idoxifene (25 n*M*), SB20 (2.5 μ*M*), and SB19 (25 n*M*) were injected and analyzed by LC–MS using different mobile phase compositions. These mobile phases were chosen to have varying aqueous compositions in order to investigate the effect of water upon the APPI response. Since water is a common component in the mobile phase for reversed-phase HPLC techniques, it was deemed important to characterize its effects upon the APPI process.

The results shown in Fig. 3A–C were obtained from the SIM APCI (upper) and APPI (lower) LC–MS analysis of a synthetic mixture containing the

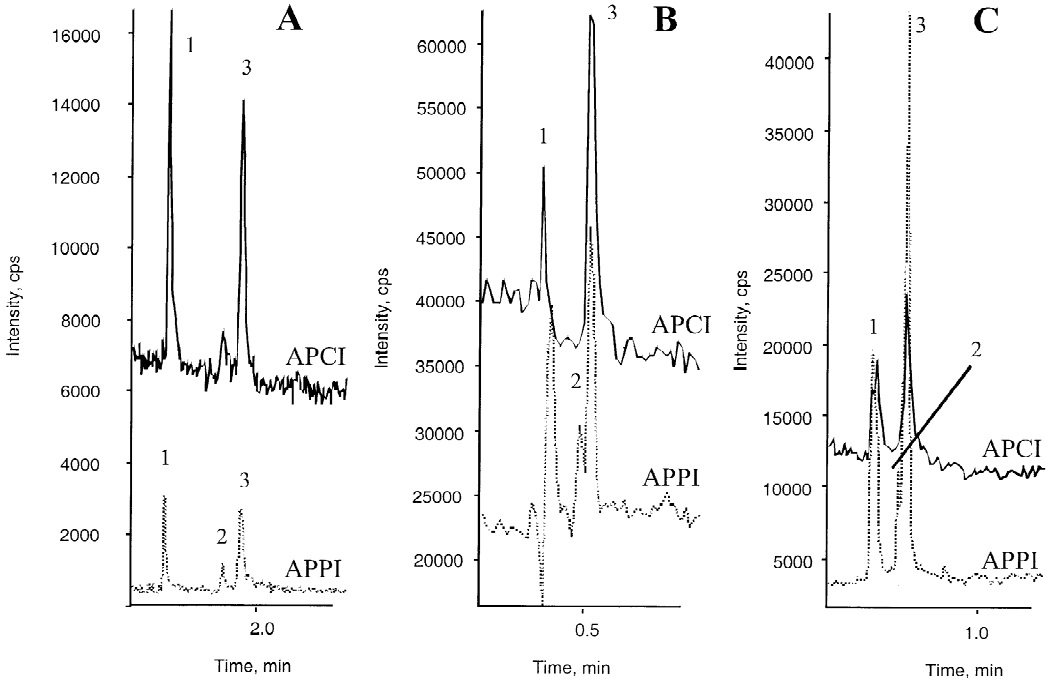


Fig. 3. Comparisons on SIM APCI LC–MS and SIM APPI LC–MS obtained from three mobile phases: (A) methanol–H_2O 85:15, v/v (1% HCOOH); (B) MeOH 99% (1% HCOOH); (C) MeOH (100%). Other LC conditions: flow-rate of mobile phase 0.7 ml/min, 10 μl sample injection. Peak identities and sample concentration in the mixture: (1) idoxifene (25 n*M*), (2) SB20 (2.5 μ*M*), (3) SB19 (25 n*M*). The SIM selected ions were *m*/*z* 524, 471, and 538 for peaks 1, 2, and 3, respectively.

idoxifene parent drug and its two metabolites using mobile phases that were composed of (A) methanol–water (85:15, v/v) (1% HCOOH), (B) methanol with the addition of 1% formic acid, and (C) 100% methanol, respectively. The peak identities and sample concentration of the mixture shown in Fig. 3 are: (1) idoxifene (2.5 μ*M*), (2) SB20 (25 μ*M*), and (3) SB19 (25 n*M*). The ions shown in the ion current profiles in Fig. 3A–C were selected at *m*/*z* 524, 471, and 538 for peaks 1, 2, and 3, respectively. The SIM APCI analyte traces (upper dark traces in Fig. 3A–C) vary by a factor of four in signal intensity (from 16 000 cycles per second (cps) in Fig. 3A to 60 000 cps in Fig. 3B) as the solvent composition is varied from 85:15 (v/v) methanol–water (1% HCOOH) to 100% methanol (Fig. 3C). In contrast, the SIM APPI analyte traces for compound 3 (lower dotted traces in Fig. 3A–C) vary by a factor of 15 in signal intensity (from 3000 cps in Fig. 3A to 45 000 cps in Fig. 3C) under corresponding solvent compositions. It should be noted that the SIM APPI signal intensity for the other two compounds in this test mixture varied in the same manner but to a lesser degree under these SIM APPI experimental conditions. Thus we conclude that in these as well as other experiments (not shown here) the SIM APPI signal intensity is diminished considerably when the mobile phase contains increasing percentages of water (Fig. 3A). Although the results shown in Fig. 3C were obtained with 100% methanol in the absence of formic acid, control experiments indicated that it was the addition of water and not formic acid that caused the variations in signal response in Fig. 3A and B.

3.3. Formation of radical cations vs. protonated molecules

The results obtained in these studies have demonstrated that SB20 can be ionized more readily by APPI than by APCI. With APPI, ions of two different mass-to-charge ratios were observed representative of the molecular species for SB20: $M^{\cdot+}$ and $(M+H)^+$. These ions represent the radical cation and the protonated molecule, respectively. The relative abundance of the radical cation and the protonated molecule varied as a function of the composition of the mobile phases as can be seen in Fig. 4A and B. In these experiments SIM APPI LC–MS analysis of

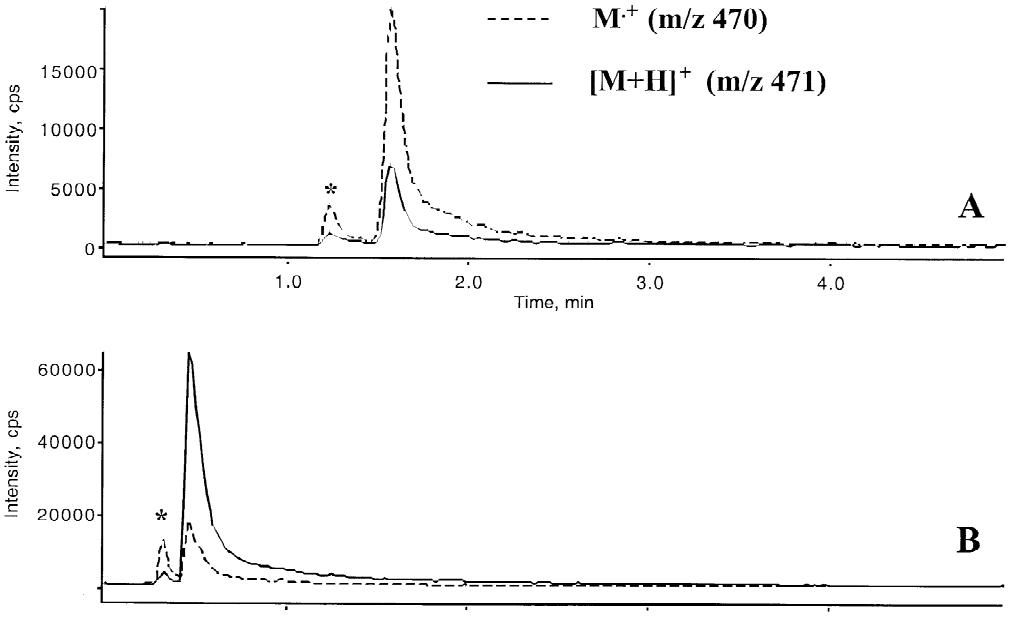


Fig. 4. APPI LC–MS SIM signal intensities of $M^{\cdot+}$ (peak in dashed line) and $(M+H)^+$ (peak in solid line) obtained from two mobile phases: (A) acetonitrile–H_2O 85:15, v/v (1% HCOOH); (B) MeOH (100%). Other conditions: MeOH (100%), flow-rate 0.7 ml/min, 10 μl sample injection. Sample concentration: SB20 10 μg/ml made up in mobile phase. An unidentified component is labeled with *.

a synthetic standard containing SB20 was recorded by monitoring both the $M^{\cdot+}$ (m/z 470) and $(M+H)^+$ (m/z 471) via SIM with two different LC mobile phases (Fig. 4A and B). The radical cation was observed in Fig. 4A to be almost four times more abundant than the $(M+H)^+$ ion in terms of peak area. In contrast, however, in Fig. 4B $(M+H)^+$ ion was considerably more abundant than the radical cation. From these data it appears the relative abundance between the radical cation (m/z 470) and the protonated molecule (m/z 471) may be significantly affected by the composition of the mobile phase. We have observed this phenomenon with other compounds (not described herein) as well using the same APPI LC–MS interface and do not currently have an explanation for this seemingly unusual behavior.

3.4. Effect of mobile phase flow-rate on sensitivity

Another important observation with the APPI interface is an apparent increase in sensitivity when the HPLC flow-rate is reduced. As shown in Fig. 5A–C, the detection sensitivity of APPI LC–MS was indeed increased with a decrease in mobile phase flow-rate. In each experiment the performance was optimized under the respective experimental conditions. The absolute signal intensity varied considerably between these SIM APPI LC–MS experiments. It is particularly noteworthy that the sensitivity for SB20 increased dramatically as the HPLC flow-rate was reduced from 0.7 to 0.1 ml/min. Under otherwise similar conditions, the detection sensitivity with a flow-rate of 0.1 ml/min was six to eight times more than with a flow-rate of 0.7 ml/min for idoxifene and SB19. However, the increase in detection sensitivity with decreased mobile phase flow-rate for the neutral compound, SB20, was much greater. It should be noted, however, that the concentration for SB20 was 100 times greater than the other two analytes in this synthetic mixture. In terms of peak area, the signal intensity of SB20 at the flow-rate of 0.1 ml/min is several thousand times greater than at the flow-rate of 0.7 ml/min. When comparing optimized APPI LC–MS results in Fig. 5 with optimized APCI LC–MS results in Fig. 3A–C,

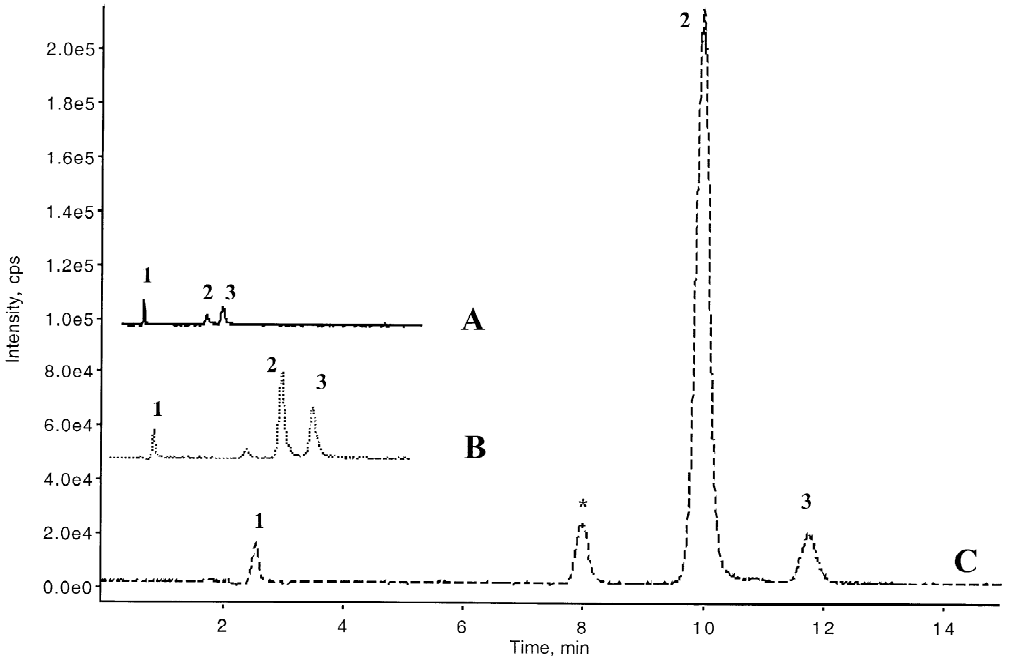


Fig. 5. Effect of flow-rate on APPI LC–MS detection sensitivity. Mobile phase flow-rates: (A) 0.7 ml/min; (B) 0.35 ml/min; (C) 0.1 ml/min. Other LC conditions: acetonitrile–H_2O 85:15 v/v (1% HCOOH), 10 μl sample injection. Peak identities and sample concentration in the mixture: (1) idoxifene (25 n*M*), (2) SB20 (2.5 μ*M*), (3) SB19 (25 n*M*).

it can be concluded that with optimized conditions APPI LC–MS can provide improved detection sensitivity when compared with APCI LC–MS for the analytes used in this study. The limit of detection for SB20 with APPI LC–MS was determined to be 100 ng/ml as a standard sample made up with the mobile phase (acetonitrile–water 85:15 (v/v) in 1% HCOOH). Although this was a significant improvement over the detection limits using either electrospray or APCI techniques, this still is not a suitable level of sensitivity for modern bioanalytical experiments.

3.5. Signal-to-noise (S/N) ratio

Fig. 6 shows a comparison of the total ion current (TIC) signal-noise-ratio (S/N) observed for the full-scan APCI LC–MS (Fig. 6A) and full-scan APPI LC–MS determination of a synthetic mixture containing idoxifene (Fig. 6B) over two different mass scan ranges. The purpose of this experiment was to compare the chemical noise and total ion current stability during a full-scan acquisition using APCI and APPI conditions. The ion current profiles shown in Fig. 6A and B are acquisitions of idoxifene from m/z 100→500 by APCI LC–MS and APPI LC–MS, respectively. As can be seen from the data within the same m/z range, APPI LC–MS has a higher S/N ratio between the idoxifene chromatographic peak (Fig. 6B) and the same chromatographic peak via APCI LC–MS (Fig. 6A). When the full scan range is reduced to m/z 200→500 for APCI LC–MS, as shown in Fig. 6C, the S/N ratio for idoxifene is improved, but is still not as high as in Fig. 6B under APPI conditions. These results suggest improved selectivity for APPI techniques for analytes of interest relative to chemical background in the system. This characteristic could afford some advantages for the analysis of biological sample extracts since the analytes of interest could perhaps be more easily detected in the presence of matrix components and other chemical constituents in the system.

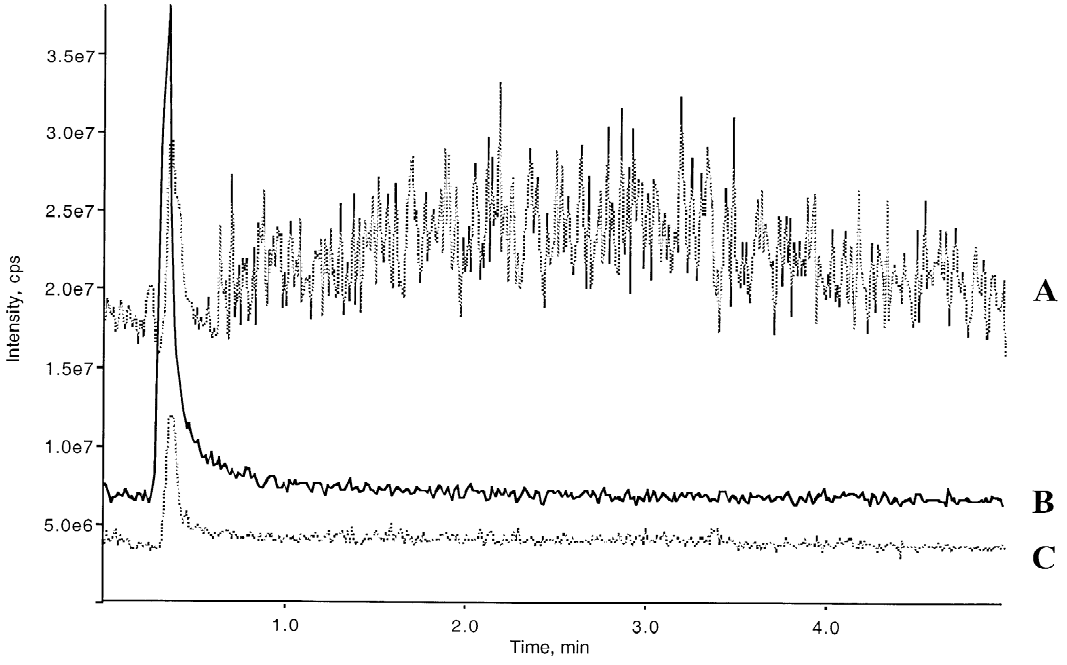


Fig. 6. APPI LC–MS and APCI LC–MS full scans on idoxifene over different ranges of m/z to demonstrate the difference in chemical background between APPI and APCI LC–MS conditions. (A) APCI LC–MS full scan (m/z 100→550); (B) APPI LC–MS full scan (m/z 100→550); (C) APCI LC–MS full scan (m/z 200→550). LC conditions: MeOH (100%), flow-rate 0.7 ml/min, 10 μl sample injection. Sample concentration: idoxifene 100 ng/ml made up in mobile phase.

3.6. Reduced matrix suppression in APPI LC–MS–MS

In electrospray mass spectrometry, matrix suppression is often observed when the matrix components from a biological sample coelute with the analyte(s). A recent report [26] has introduced a method to investigate this matrix suppression. The method was adopted in this paper to investigate the matrix suppression for APPI LC–MS. The SRM LC–MS ion current profiles shown in Fig. 7 display the data acquired over a 12-min period, since no chromatographic peaks were observed beyond this time window. The perturbation of the ion current trace due to the injector actuation is observed as a spike at 2.0 min. The corresponding large negative dips in the ion current traces observed for both the solvent blank (A) and the negative control plasma extract (B) were observed at approximately 4. 3 min and are essentially the same. The observed negative deflection at the column void even from injection of mobile phase has been observed in related experiments (unpublished results) and the literature [26]. The absence of any negative deflection of the ion current trace in Fig. 7 (trace B) after the column void suggest that chromatographic elution of endogenous components do not cause any detectable matrix suppression under these APPI LC–MS conditions. This could result from the ionization potential (IP) of the matrix components being sufficiently higher than the analytes such that the matrix is not ionized or does not interfere in the photoionization process. However, more detailed experiments are required to validate this premise.

3.7. SRM APPI LC–MS of idoxifene and its major metabolite in human plasma

Fig. 8A–D shows the SRM APPI LC–MS traces for the analysis of control human plasma sample. Panels A, B, C, and D shown in Fig. 8 show the double blank, blank (d_5-idoxifene, 250 ng/ml, m/z 529→98), idoxifene (10 ng/ml, m/z 524→98), and SB19 (25 ng/ml, m/z 538→112), respectively. At

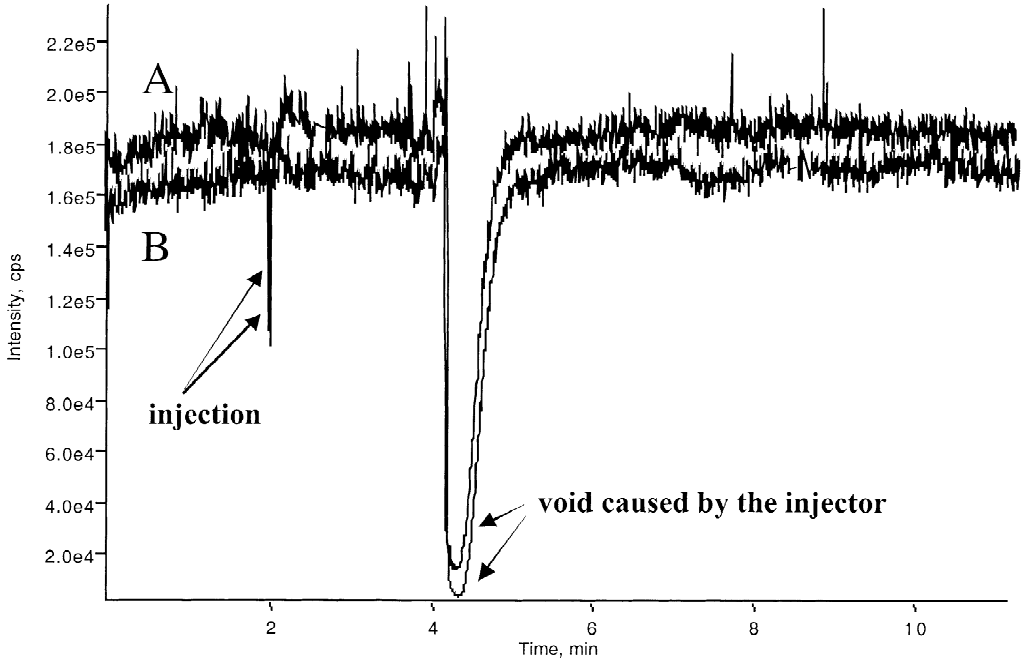


Fig. 7. SRM of SB19 (transition m/z 538→112) to investigate the matrix suppression effect under APPI LC–MS–MS conditions. (A) Injection of 10 μl of mobile phase; (B) injection of 10 μl of blank plasma extract. Other LC conditions: acetonitrile–H_2O 85:15 v/v (1% HCOOH), flow-rate 0.1 ml/min. Post-column infusion of SB19 (10 μg/ml) at 5 μl/min.

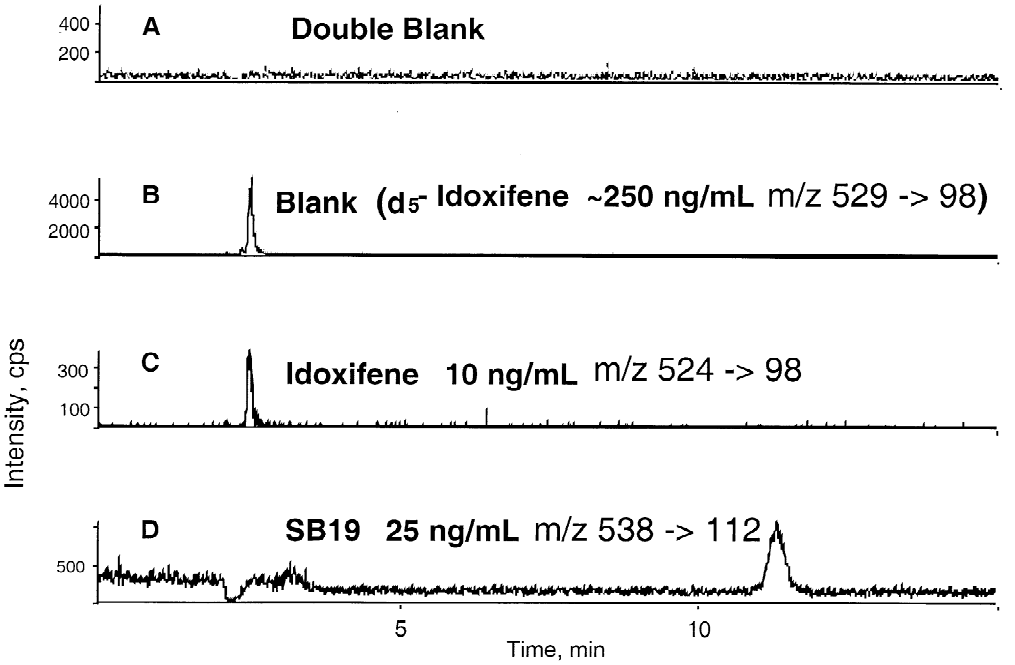


Fig. 8. SRM APPI LC–MS–MS traces of control human plasma extract spiked at LOQ. (A) Double blank; (B) blank (internal standard d_5-idoxifene at 250 ng/ml, transition m/z 529→98); (C) idoxifene at LOQ (10 ng/ml, transition m/z 524→98); (D) SB19 at LOQ (25 ng/ml, transition m/z 538→112). Other LC conditions: acetonitrile–H_2O 85:15 v/v (1% HCOOH), flow-rate 0.1 ml/min, 10 μl sample injection.

the early stages of these experiments, attempts were made to include the SRM APPI LC–MS quantitation of the neutral metabolite, SB20. However, SB20 could not be quantified at clinically relevant levels in human plasma, so it was excluded from these studies.

Calibration curves were plotted for both idoxifene and SB19, based on the established SRM LC–MS quantitation approach as reported earlier [22]. For idoxifene, a linear relationship (correlation coefficient=0.993) was found within the linear range of 10–500 ng/ml. For SB19, a linear relationship (correlation coefficient=0.990) was found within the linear range of 25–500 ng/ml. Both of these calibration curves were determined using d_5-idoxifene as internal standard for each analyte. Although the determined dynamic ranges for the parent drug and the major metabolite were not particularly wide, these ranges satisfy the needs of the method for determining idoxifine and its major metabolite in human plasma.

4. Conclusions

The results obtained in this work have demonstrated some potential advantages for APPI as an ionization approach to increase the detection sensitivity for LC–MS analysis. These include an apparent insensitivity to matrix suppression of ionization and relatively high signal-to-noise ratios between analyte ion current and the background chemical noise in the system. Also, if reduced HPLC flow-rates are employed improved sensitivity for otherwise intractable analytes such as the neutral metabolite described in this report may be obtained. In the neutral metabolite example cited a 100 ng/ml detection limit was achieved when neither electrospray nor APCI could produce any ion current from this compound at this level. However, this performance was still not adequate for typical bioanalytical determination of this compound in biological samples.

The described APPI LC–MS interface also ap-

pears to have some potential limitations at least in the context of bioanalytical LC–MS–MS applications. Increasing percentages of water in the HPLC mobile phase appears to reduce the sensitivity of the technique. This could cause some limitations in reversed-phase HPLC applications where sometimes high percentages of an aqueous composition are needed to affect a satisfactory chromatographic separation.

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