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ORIGINAL ARTICLE

Chromatographic behavior of co-eluted plasma compounds and effect on screening of drugs by APCI-LC–MS(/MS): Applications to selected cardiovascular drugs

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Abstract Chromatographic behavior of co-eluted compounds from un-extracted drug-free plasma samples was studied by LC–MS and LC–MS/MS with positive APCI. Under soft gradient, total ion chromatogram (TIC) consisted of two major peaks separated by a constant lower intensity region. Early peak (0.15–0.4 min) belongs to polar plasma compounds and consisted of smaller mass ions ($m/z < 250$); late peak (3.6–4.6 min) belongs to thermally unstable phospholipids and consisted of fragments with $m/z < 300$. Late peak is more sensitive to variations in chromatographic and MS parameters. Screening of most targeted cardiovascular drugs at levels lower than 50 ng/mL has been possible by LC–MS for drugs with retention factors larger than three. Matrix effects and recovery, at 20 and 200 ng/mL, were evaluated for spiked plasma samples with 15 cardiovascular drugs, by MRM–LC–MS/MS. Average recoveries were above 90% and matrix effects expressed as percent matrix factor (% MF) were above 100%, indicating enhancement character for APCI. Large uncertainties were significant for drugs with smaller masses ($m/z < 250$) and retention factors lower than two.

1. Introduction

Screening, identification and quantification of drugs in biological samples remain a continuous challenging task for bio-analysis. Analysis of a large number of samples in short time (pharmaco-kinetic studies) and screening for a large number of drugs and metabolites in small samples (systematic toxicological analysis

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(STA)) necessitate continuous development of methods with adequate sensitivity, selectivity and short run time.

Methods, based on LC–MS and LC–MS/MS are currently the methods of choice for pharmaceutical and clinical analysis due to high sensitivity and selectivity. A most reliable method combines optimized extraction with careful selection of LC and MS parameters.

Recent advances in LC–MS(/MS) were reviewed by many authors [1–3]. Peters [1] provided an overview on recent developments of LC–MS(/MS)-based analysis in clinical and forensic toxicology focusing on STA and multi-analyte procedures. He concluded that hybrid mass spectrometers and high resolution time of flight mass spectrometers (TOF-MS) are essential to generating rich product ion spectra that can be searched against libraries of reference spectra, and for screening of compounds without reference standards. Maurer [2] reviewed multi-analyte procedures for screening and quantification of drugs in blood and plasma by LC–MS and LC–MS/MS. He concluded that cost issues and irreproducibility of ionization are still limitations for LC–MS/MS to become the gold technique in clinical and toxicological and doping control. Numerous methods for targeted analytes such as amphetamines, opiates and therapeutic drugs were reported [4–9]. Gonzalez et al. [4] reported an LC–MS/MS method for quantification of 55 drugs prescribed in combined cardiovascular therapy. Krichherr et al. [5] reported quantitative analysis of 48 antidepressant and antipsychotics in serum by LC–MS/MS. Matrix effects from co-eluted endogenous sample compounds were addressed in all previous reviews and methods.

Matrix effect (ME) is defined as the direct and indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the sample [10,11]. In LC–MS/MS methods, ionization and non-ionization matrix effects are familiar. Ionization matrix effects refer to the ionization suppression or enhancement caused by co-eluted extracted substances from the biological matrix [12]. Non-ionization matrix effect refers to mainly loss of analyte during sample preparation and separation steps. Ionization matrix effects dominate assuming an efficient extraction method (clean up) preceded injection of the sample; however, non-ionization matrix effects should not be ignored, especially when un-extracted samples were employed [12].

Perspectives in addressing matrix effects, and recovery issues in regulated drug bioanalysis by LC–MS/MS were reviewed [12,13]. Liang [13] examined key issues related to ionization matrix effects and evaluated various methods to address matrix effect problems with effectiveness and practicality. Huang et al. [12] emphasized that in LC–MS/MS methods, the cause of assay bias from sources of matrix is mainly due to an ionization change; however, extraction recovery may cause analytical assay variation. He proposed using the term "matrix variation" rather than ME.

The Crystal City conference report [14] and the European Medicines Agency final bioanalysis method validation (BMV) guidance [11] adapted matrix factor (MF), defined as a ratio of the analyte peak response in the presence of matrix ions (post-spiked) to the analyte response in the absence of matrix ions (neat standards), for quantitative measurements of ionization ME. Matuszewski et al. [15] suggested ME (%) = 100 × MF. An ME of 100 implies no suppression or enhancement matrix effects ionization. A value either less or more than 100 suggests either ionization suppression or enhancement.

Recovery assessment (non-ionization matrix effects) usually accompanies ME assessment, even with un-extracted samples. FDA guidance, defines recovery as the percentage ratio of detector response of pre-spiked blank versus that of post-spiked blank. Pre-spiked analyte refers to analyte spiked into blank plasma before extraction, while, post-spiked analyte refers to analyte spiked into blank plasma extract.

Matrix effects evaluation and reduction was the subject of many papers. [16–20]. Chambers et al. [16] presented systematic comprehensive strategy that optimizes sample preparation and chromatography to minimize matrix effects in bioanalytical LC–MS/MS analysis. Van Eeckhaut et al. [17] reviewed the assessment, reduction and evaluation of matrix effects during validation of bioanalytical methods. They emphasized ionization effects as the main reason for matrix effects. They recommended that evaluation of matrix effect should be a mandatory part of the validation procedure for all LC–MS based methods. Matrix effect studies in most published papers were performed on extracted plasma samples in ESI mode and in conjunction with targeted analytes. To our knowledge, few publications dealt with the behavior and matrix effect of plasma endogenous compounds only [21,22].

In this work we studied the chromatographic behavior of co-eluted compounds from un-extracted plasma samples and their effects on screening of drugs by LC–MS(/MS) with atmospheric pressure chemical ionization (APCI). Our results emphasized the dependence of matrix effects on *m/z* of molecular ions and retention factors of drugs. Eluted drugs with retention factors larger than three could be screened by single stage LC–MS at levels lower than 50 ng/mL. Lower matrix effects and larger recoveries were observed for drugs with molecular ions *m/z* larger 300 and/or retention factors larger than 3 under MS/MS conditions.

2. Experimental

2.1. Chemicals and reagents

Studied drugs presented in Tables 1 and 2 were generously provided by International Pharmaceutical Research Center (IPRC) (Amman, Jordan). HPLC-grade acetonitrile, formic acid, ammonium formate and other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Water purified by the use of Millipore Milli-Q system (Lemount, Switzerland) was used for preparation of all solutions. Six different batches of drug-free plasma from healthy volunteers (3 males and 3 females; age between 18 and 25 years) were obtained from King Abdullah Hospital (Irbid, Jordan).

2.2. LC–MS/MS apparatus and conditions

HPLC analysis was carried out on an Agilent 1200 series (Palo Alto, CA, USA) system consisting of a quaternary pump and vacuum degasser and a refrigerated autosampler (5 °C). Chromatography was performed on a 50 mm × 4.6 mm, 5-μm particle (chromatographic behavior) and 50 mm × 2.1 mm, 5-μm particle (ME and recovery) Agilent C_{18} columns at room temperature. The mobile phase consisting of solvent A (0.1% (v/v) aqueous formic acid with 1.0 mM ammonium formate) and solvent B (0.1% (v/v) formic acid with 1.0 mM ammonium formate in acetonitrile) was delivered at a flow rate of 0.60 mL/min. The applied LC gradient was the following: 0–1.0 min 20% B; 1–5.0 min 20–95% B, 5.0–7.0 min 95% B, 7.0–7.5 min 95–20% B, 7.5–9 min 20% B.

Detection was carried out using an API 3200 triple quadrupole tandem mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with a Turbo V source to produce ions from liquid samples. The Turbo V source can use the TurboIonSpray probe (ESI) or the APCI probe. The APCI probe source was operated in the positive mode at

Table 1 Retention times and signal-to-noise ratios (*S/N*) for eight drugs separated by LC–MS in post-spiked plasma sample (50 ng/mL of each drug).

Drug	Molecular ion $(M+H)^+$	Retention time (min)	Signal-to-noise ratio (*S/N*)		LOQ (ng/mL)
			TIC	XIC	
Propranolol	260.3	4.16	–	87.5	6.2
Gliclazide	324.2	5.25	20.3	60.7	8.3
Enalapril	377.2	4.23	–	45.6	10.8
Ramipril	417.2	4.60	12.5	75.3	7.1
Rosuvastatin	482.1	4.98	45.3	68.3	7.7
Glimepiride	491.2	5.65	6.5	25.3	17.5
Atorvastatin	559.6	5.54	17.5	71.2	7.2
Cand. Cilex.	611.3	6.23	18.7	75.3	7.1

TIC = total ion chromatogram. XIC = extracted ion chromatogram. LOQ = limit of quantitation.

Table 2 Percentage matrix effects (ME) and extraction recovery for studied drugs and their MRM analysis conditions.[a]

Drug	MRM transition	Conc. (ng/mL)	Retention time (min)	Matrix effect (%) (mean ± SD)	Recovery (%) (mean ± SD)
Metformin	130.1 → 71.1	20	0.28	150.1 ± 6.8	78.5 ± 10.8
		200		145.6 ± 3.4	93.2 ± 6.5
Aspirin	181.2 → 91.2	20	0.32	147.6 ± 9.8	86.7 ± 9.5
		200		145.6 ± 6.7	93.6 ± 4.5
Propranolol	260.3 → 155.2	20	3.99	96.3 ± 5.6	95.3 ± 5.9
		200		95.7 ± 2.3	94.3 ± 4.9
Trimethoprim	267.2 → 166.1	20	0.32	132.3 ± 9.8	89.6 ± 6.5
		200		128.6 ± 6.7	91.3 ± 3.8
Gliclazide	324.3 → 127.2	20	5.07	118.2 ± 6.7	87.6 ± 7.5
		200		113.5 ± 5.2	91.3 ± 4.5
Enalapril	377.2 → 234.2	20	4.01	98.6 ± 5.7	110.2 ± 11.3
		200		103.2 ± 2.5	106.7 ± 9.5
Lisinopril	406.2 → 246.2	20	0.35	147.3 ± 15.3	75.3 ± 9.5
		200		140.8 ± 9.6	81.6 ± 7.8
Ramipril	417.3 → 234.3	20	4.33	98.6 ± 3.4	87.6 ± 6.6
		200		99.4 ± 2.8	97.6 ± 4.3
Valsartan	436.2 → 207.2	20	5.07	106.8 ± 9.5	97.6 ± 4.3
		200		96.8 ± 4.6	93.4 ± 5.2
Rosuvastatin	482.1 → 258.3	20	4.79	126.3 ± 7.5	95.6 ± 4.5
		200		118.9 ± 4.8	97.2 ± 2.3
Glimepiride	491.4 → 126.3	20	5.46	136.0 ± 9.5	104.4 ± 9.8
		200		128.6 ± 7.5	104.4 ± 6.5
Glipizide	494.3 → 169.2	20	5.38	129.4 ± 6.7	89.7 ± 6.9
		200		118.3 ± 5.4	91.3 ± 2.6
Telmisartan	515.2 → 497.3	20	4.44	117.5 ± 6.8	97.6 ± 2.8
		200		114.3 ± 3.9	94.2 ± 4.2
Atorvastatin	559.6 → 440.3	20	5.35	128.6 ± 11.5	89.6 ± 6.6
		200		122.4 ± 7.5	91.4 ± 3.8
Cand. Cilex.	611.3 → 423.5	20	6.06	126.8 ± 8.9	91.3 ± 9.8
		200		121.4 ± 6.6	96.4 ± 6.5

[a]Five replicates at each nominal concentration

450 °C with a needle current of 4.0 μA. Nitrogen was used as curtain gas (CUR) (10 psi) and gas 1 (70 psi). Analysis was performed by either Q1-scan (MS) mode or multiple reaction monitoring (MRM) mode (MS/MS). For Q1-scan mode declustering potential (DP) was set at 50 V, entrance potential (EP) 5 V and collision entrance potential (CEP) 20 V. Additionally, for MRM transitions, collision gas (CAD) was set at 6 psi, collision energy (CE) 35 V, collision cell exit potential (CXP) 3.0 V and dwell time 50 ms for each MRM transition and 5 ms pause. Data processing and system control were performed by Analyst 1.5 software (Applied Biosystems).

2.3. *Standard solutions*

Standard stock solutions of 0.50 mg/mL of each drug in Tables 1 and 2 were prepared separately in methanol. For LC–MS experiments, a stock solution mixture (5.00 μg/mL) of drugs in Table 1 was prepared by transferring 100 μL of each drug solution into a 10 mL volumetric flask, then diluting to the mark with 20% acetonitrile solution. A working solution mixture (200 ng/mL) was prepared daily from the stock solution mixture by sub-sequent dilution with 20% actonitrile solution. For LC–MS/MS experiments, a stock solution mixture (5.00 μg/mL) of drugs in Table 2 was prepared by the same way as in LC–MS. Working solution mixtures (80 and 800 ng/mL) were prepared daily from the stock solution mixture by sub-sequent dilution with 20% actonitrile solution.

2.4. *Sample preparation*

Plasma was prepared from whole blood as follows: Blood samples were collected into tubes containing EDTA; then centrifuged at 1.3*g* for 10 min at 4 °C. The plasma supernatant was carefully separated from blood cells and collected in polypropylene tubes to be frozen at −20 °C until analysis [4].

Blank solution was prepared by transferring 200 μL drug-free human plasma to a 2.00 mL Eppendrof tube, followed by 800 μL of 20% acetonitrile solution, vortexed for 30 s, and the slightly turbid solution was centrifuged at 13,000 rpm for 5 min. Pre-spiked solutions were prepared by transferring 200 μL drug-free human plasma to a 2.00 mL Eppendrof tube, followed by 50 μL of drug mixture, vortexed for 30 s, followed by 750 μL of 20% acetonitrile solution, vortexed for 30 s, and the slightly turbid solution was centrifuged at 13,000 rpm for 5 min. Post-spiked solutions were prepared by transferring 200 μL drug-free human plasma to a 2.00 mL Eppendrof tube, followed by 750 μL of 20% acetonitrile solution, vortexed and centrifuged in the same manner. To supernatant 50 μL of drug mixture was added and vortexed again for 30 s. Nominal concentrations were calculated based on the volume of plasma (200 μL). Neat standards were prepared by diluting 50 μL of working standards to 1.00 mL with 20% acetonitrile solution. Injected concentrations were five times lower than nominal concentrations. A 200 μL of each solution was transferred into a clean 96-deep well plate and 10 μL were injected into the LC–MS/MS system.

2.5. *Matrix effects and recovery evaluation*

Matrix effects and recovery for each spiked drug in the mixture were determined at two nominal concentrations (20 and 200 ng/mL). Analyte concentrations were selected to contain reported concentrations for clinical studies.

Recovery (or extraction efficiency) was determined by measuring the response of an extracted sample (pre-spiked) against the response of a post-extracted spiked sample:

$$\text{Recovery (\%)} = \left(\frac{C}{B}\right) \times 100$$

Matrix effects (ME) was measured by referring the response of a post-spiked sample to the response of a neat standard (non-extracted neat sample):

$$\text{ME (\%)} = \left(\frac{B}{A}\right) \times 100$$

where A is the response of neat standards, B is the response of post-spiked samples and C is the response of pre-spiked samples. These equations are similar to those reported by Matuszewski et al. [15].

3. Results

The TIC–LC–MS chromatogram for a drug-free plasma sample under conditions specified in experimental section is presented in Fig. 1A. Co-eluted plasma compounds were distributed over two major peaks, separated by a middle region with relatively steady background level. The early peak representing polar compounds was eluted at $t_{r1}=0.60$ min with width at half maxima, $W_{1/2}(1)=0.20$ min. The late peak representing semi- and non-polar compounds was eluted at t_{r2} (average)$=4.24$ min with $W_{1/2}(2)=0.90$ min. When flow rate was increased from 0.60 mL/min to 1.0 mL/min (Fig. 1B), both retention times were decreased ($t_{r1}=0.37$, $t_{r2}=3.20$ min). When acetonitrile was replaced by methanol in mobile phase (Fig. 1C), the early peak was not affected, while retention time and broadness of late peak were significantly increased ($t_{r2}=6.20$ min, $W_{1/2}(2)=1.20$ min). When pH of the mobile phase was increased from 2.87 (0.1% formic acid) to 4.50 (10 mM ammonium formate) (Fig. 1D), the early peak was not affected, while retention time for the late peak increased from 4.24 to 5.06 min. No significant change in either retention times or broadness of both peaks was observed when column temperature was raised from ambient to 40 °C (Fig. 1E). A significant broadness of early peak was observed when the APCI probe temperature was decreased from 450 °C to 400 °C (Fig. 1F). When a hard gradient was applied (0–1.0 min 70% B, 1–5.0 min 70–95% B, 5.0–7.0 min 95% B, 7.0–7.5 min 95–70% B, 7.5–9 min 70% B) (Fig. 1G), a large decrease in retention time of late peak was observed ($t_{r2}=1.94$ min). When the 50 mm × 4.6 mm diameter column was replaced by a 50 mm × 2.1 mm diameter column, a tremendous decrease in retention time and broadness of both peaks was observed (Fig. 1H) ($t_{r1}=0.20$ min, $W_{1/2}(1)=0.10$ min; $t_{r2}=3.76$ min, $W_{1/2}(2)=0.25$ min). Thus, for subsequent studies a 50 mm × 2.1 mm diameter column was employed.

Fig. 2 presents selected APCI mass spectra from selected regions of Fig. 1H. The representative MS of early peak at 0.232 min (Fig. 2A) consists of ions with $m/z<250$, which are attributed to co-eluting polar plasma compounds. Drugs such as metformin and aspirin elute in this region. Thus, these drugs could not be determined by LC–MS. Also, when determined by MRM–LC–MS/MS they are expected to suffer larger matrix effects (Table 2). However, most therapeutic drugs have molecular ions m/z $(M+H)^+>250$ or elute at retention times larger than 0.30 min. The MS representing middle region at 1.947 min (Fig. 2B) has the same ion profile as of Fig. 2A, but with ion intensities 10 times less than in Fig. 2A. The MS shown in Fig. 2C at 3.773 min is a representative of phospholipids late peak. Even phospholipids have

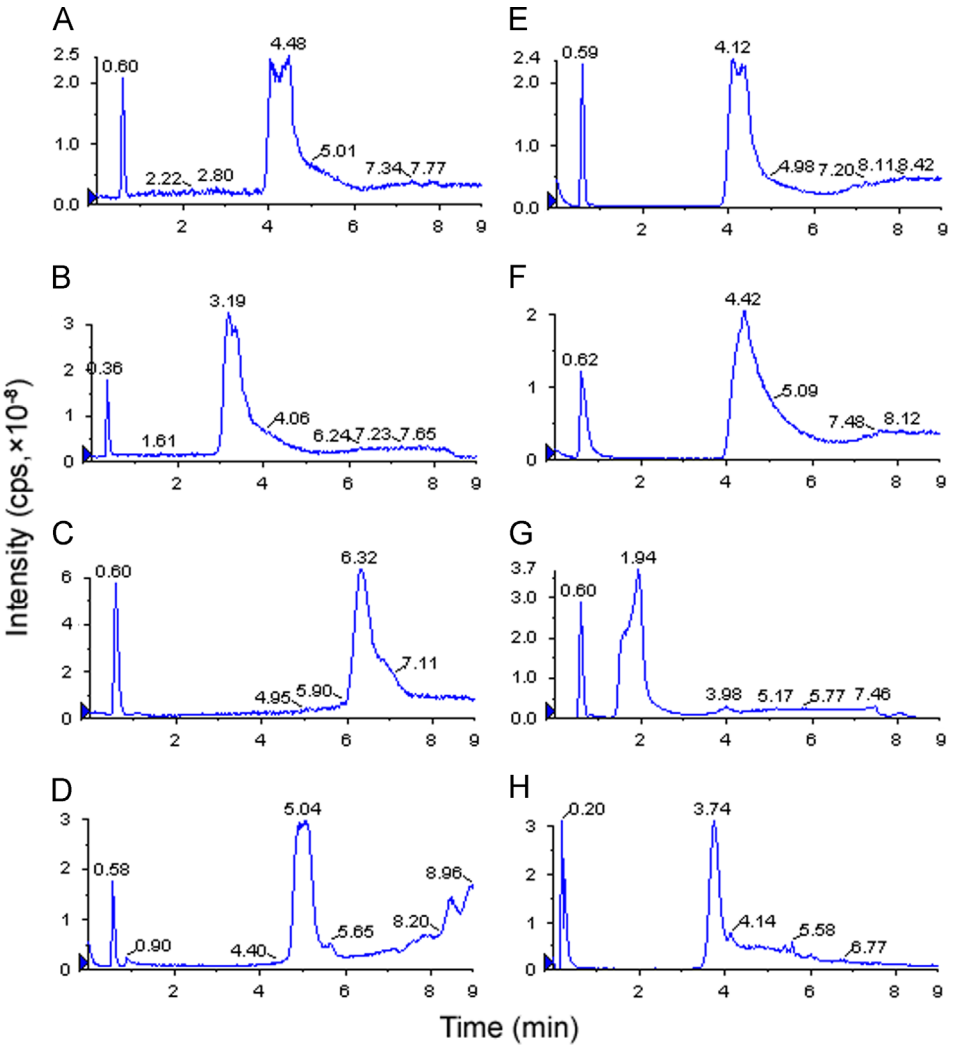

Fig. 1 (A) TIC–LC–MS chromatogram for drug-free plasma collected under: Gradient: 0–1.0 min 20% B, 1–5.0 min 20–95% B, 5.0–7.0 min 95% B, 7.0–7.5 min 95–20% B, 7.5–9 min 20% B; flow rate 0.60 mL/min; pH = 2.87; temperature: ambient; APCI probe temperature = 450 °C; column: 50 mm × 4.6 mm, 5-μm particle. (B) The TIC–LC–MS chromatogram upon increasing flow rate to 1.0 mL/min. (C) The TIC–LC–MS chromatogram upon replacing acetonitrile with methanol. (D) The TIC–LC–MS chromatogram upon increasing pH from 2.87 to 4.5. (E) The TIC–LC–MS chromatogram upon increasing column temperature from ambient to 40 °C. (F) The TIC–LC–MS chromatogram upon decreasing APCI probe temperature from 450 to 400 °C. (G) The TIC–LC–MS chromatogram upon employing a gradient: 0–1.0 min 70% B, 1–5.0 min 70–95% B, 5.0–7.0 min 95% B, 7.0–7.5 min 95–70% B, 7.5–9 min 70% B. (H) The TIC–LC–MS chromatogram upon replacing the 50 mm × 4.6 mm, 5-μm particle column by a 50 mm × 2.1 mm, 5-μm particle column.

large molar masses $m/z > 500$, the MS consists of ions with $m/z < 300$ indicating thermal instabilities of phospholipids under APCI. Thus, drugs eluting in this region usually have molecular ions m/z $(M+H)^+ > 300$ and could be easily screened by single LC–MS. Similar MS collected under ESI is much complicated and consists of ions with $m/z > 500$ with relatively high intensities [13].

Fig. 3A presents a TIC–LC–MS chromatogram for eight therapeutic drugs spiked at 50 ng/mL each. These drugs are usually prescribed for patients with cardiovascular diseases such as diabetes and hypertension. Extracted ion chromatograms (XIC)–LC–MS chromatograms at corresponding molecular ions are shown in Fig. 3B.

Table 1 presents retention time and signal-to-noise ratio (*S/N*) for each drug calculated from TIC chromatogram and corresponding XIC. *S/N* ratios from XICs, as expected, are much higher and LOQs are much lower. However, TIC peaks usually contain major fragment ions in addition to molecular ion, which may help in identification in non-targeted screening. *S/N* ratios from TIC for propranolol and enalapril could not be calculated due to overlapping between their TIC peaks. Limits of quantitation (LOQs) calculated from XICs varied between 6.2 ng/mL for propranolol and 17.5 ng/mL for glimepiride.

Fig. 4 presents MRM–LC–MS/MS chromatograms for 15 drugs spiked to a drug-free plasma sample at 20 ng/mL each. These drugs include the eight drugs studied in LC–MS (Fig. 3, Table 1). Eluted drugs have retention times either similar to early plasma peak (4 drugs) or late plasma peak (8 drugs). Table 2 presents MRM transitions, % ME and % recovery at two nominal concentrations 20 and 200 ng/mL. Most drugs show % ME larger than 100, indicating enhancement character of APCI. Recoveries of most tested drugs are around 100%, which is expected since sample preparation was dilution of plasma sample with 20% acetonitrile solution (initial composition of mobile phase) with a ratio 1:4.

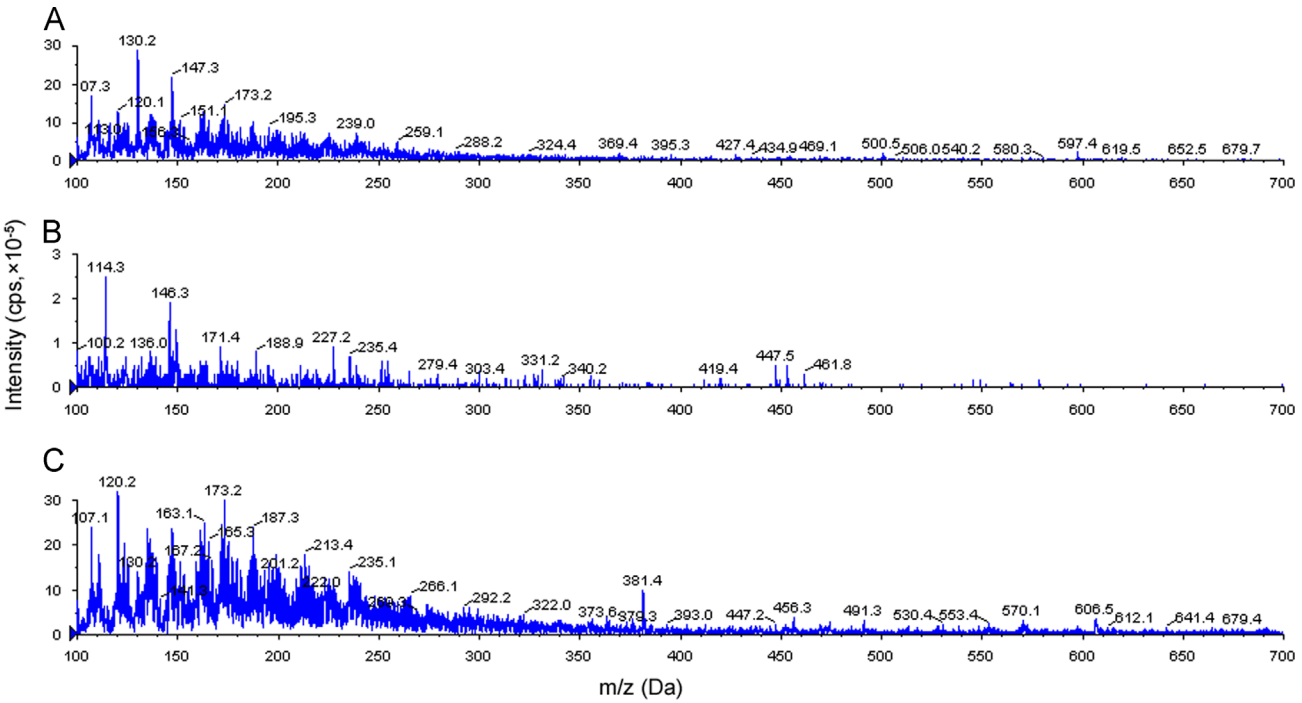

Fig. 2 Mass spectra collected from drug-free plasma sample TIC–LC–MS chromatogram (Fig. 1H) at (A) 0.232 min (early peak), (B) 1.947 min (middle region) and (C) 3.773 min (late peak).

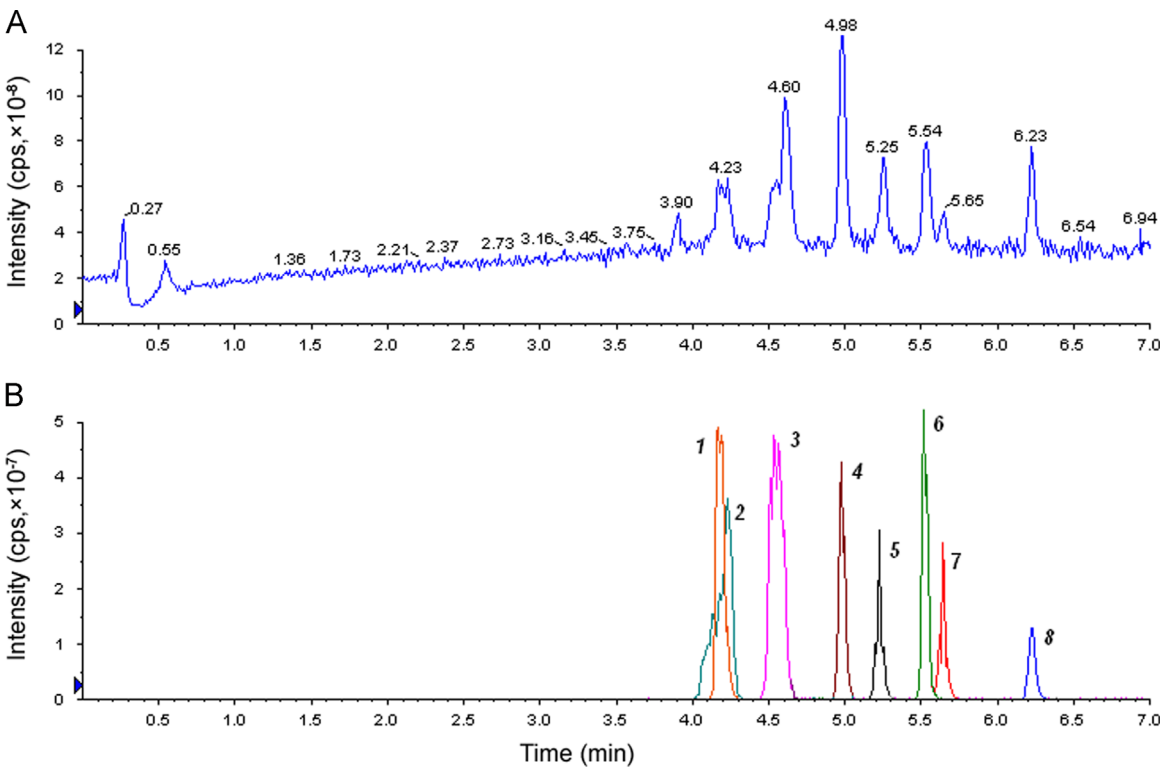

Fig. 3 (A) TIC–LC–MS chromatogram for drug-free plasma sample post-spiked with eight drugs (50 ng/mL) each as follows: 1. Propranolol; 2. Enalapril; 3. Ramipril; 4. Rosuvastatin; 5. Gliclazide; 6. Atorvastatin; 7. Glimepiride; 8. Candesartan Cilexetil. (B) XIC chromatograms for the eight drugs (overlaid) from the TIC chromatogram. Molecular ions are presented in Table 1.

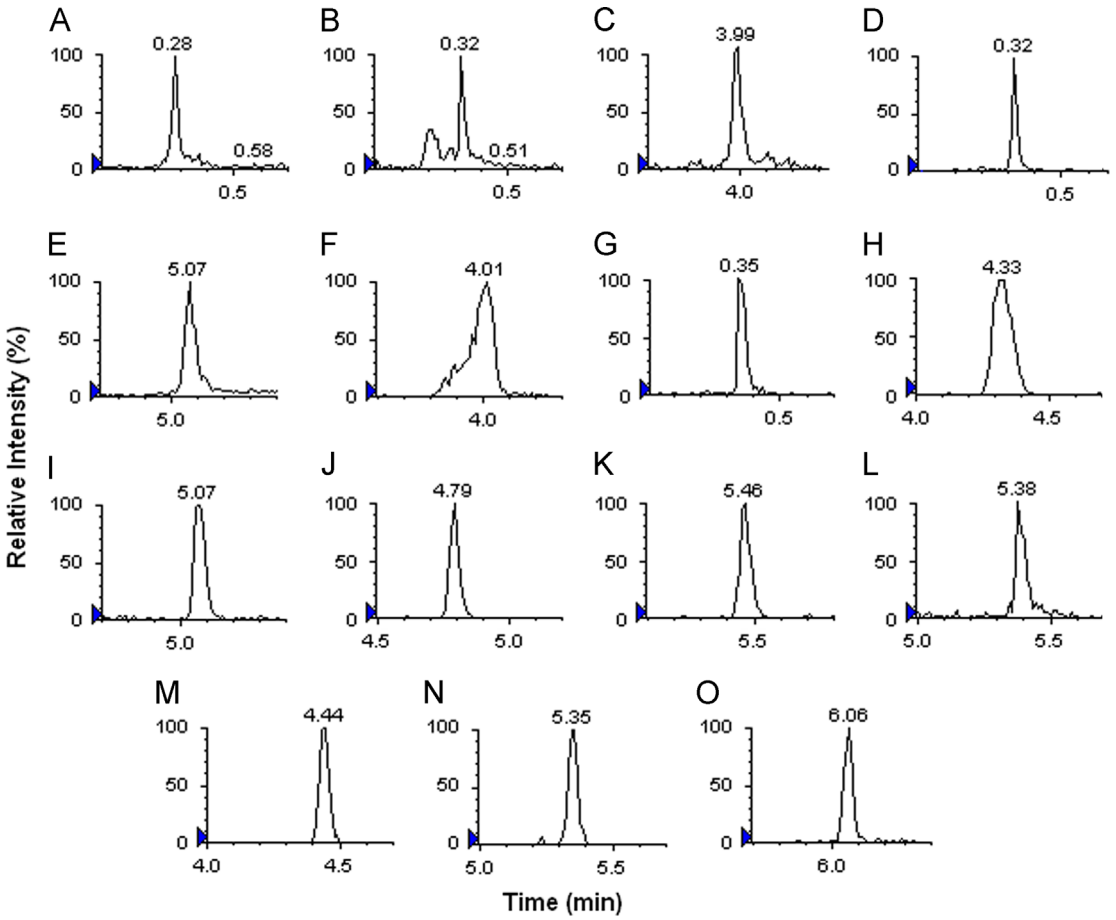

Fig. 4 MRM–LC–MS/MS chromatograms for drug-free plasma sample post-spiked with fifteen drugs (20 ng/mL) each as follows: A. Metformin; B. Aspirin; C. Propranolol; D. Trimethoprim; E. Gliclazide; F. Enalapril; G. Lisinopril; H. Ramipril; I. Valsartan; J. Rosuvastatin; K. Glimepiride; L. Glipizide; M. Telmisartan; N. Atorvastatin; O. Candesartan Cilexetil. Transitions are summarized in Table 2.

A selectivity study on six different drug-free plasma samples from healthy volunteers was performed. The MRM chromatograms did not show interfering peaks within retention times of selected drugs.

4. Discussion

To give this study a perspective, the following points have to be emphasized: Our major objective was to study the chromatographic behavior of co-eluting plasma compounds and their effects on screening of drugs under APCI-LC–MS(/MS). Thus, the sample preparation procedure was simple and just mixing plasma sample with initial concentration of mobile phase gradient to prevent precipitation inside the column. The choice of short RP column (50 mm) is similar to most published methods for drugs screening, and to allow maximum co-elution of plasma compounds within reasonable run-time. Selection of drugs for matrix effects and recovery studies considered coverage of a wide range of molecular ions and retention factors. Also, we acknowledge that APCI is not the first choice in bio-analysis and most of the applications are using ESI accompanied with a proper cleanup method.

Matrix effects of co-eluting plasma compounds are considered the major setback on LC–MS/MS methods for screening of residues of drugs in plasma. Lack of selectivity in ionization process between analytes, mobile phase constituents and co-eluting matrix compounds created non-linearity and irreproducibility problems due to suppression and enhancement in analytical signals [1–3]. Recent reported analytical methods were devoted to reduction and compensation of matrix effects including optimization of novel extraction methods and employment of sophisticated expensive MS systems.

Our results indicated that understanding the chromatographic behavior of co-eluting matrix components could help in screening and determination of many classes of drugs, especially for targeted drugs, with minimum sample preparation under APCI.

APCI produces small mass fragments (Fig. 2A–C), indicating thermal instability of plasma compounds. The elution profile of plasma compounds includes a region with low ion intensity between the polar and non-polar peaks. Chromatographic conditions could be optimized to elute analytes in this region where they are expected to experience higher recovery and lower matrix effects. Single stage LC–MS (Q1 scan) methods could be employed to screen most of popular therapeutic and illicit drugs at moderate concentrations (<50 ng/mL). Acceptable matrix effect and recovery results were obtained for the 15 drugs analyzed by MRM–LC–MS/MS over a wide concentration range of 20–200 ng/mL (Table 2).

Our results also supported enhancement character of APCI [23], which is believed that co-eluting plasma compounds stabilize analyte molecular ion and minimize its fragmentation before going to Q2 for collision induced dissociation.

Even the objectives of this study were achieved, a proper extraction method is still recommended to keep good performance of separation column for longer periods and to avoid cross-contamination, especially for screening a large number of drugs at lower concentrations.

5. Conclusions

This paper presented a systematic study of behavior of co-eluting plasma compounds and their effect on determination of drugs by APCI-LC–MS(/MS). The LC–MS chromatogram of drug-free plasma was simple and consisted of two major peaks separated by a region composed of low-intensity small mass ions. A case study of spiking fifteen selected drugs with wide range of protonated molecular ions, $(M+H)^+$, and retention factors provided acceptable recovery and matrix effects results by LC–MS/MS in MRM mode, and the possibility of their screening at moderate concentrations (<50 ng/mL) by XIC–LC–MS.

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