Comparison of ESI– and APCI–LC–MS/MS methods: A case study of levonorgestrel in human plasma

Rulin Wang, Lin Zhang, Zunjian Zhang, Yuan Tian

Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing 210009, China
State Key Laboratory of Natural Medicine, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Keywords:
Levonorgestrel
LC–MS/MS
Pharmacokinetics
Electrospray ionization
Atmospheric pressure chemical ionization

ABSTRACT

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) techniques for liquid chromatography–tandem mass spectrometry (LC–MS/MS) determination of levonorgestrel were evaluated. In consideration of difference in ionization mechanism, the two ionization sources were compared in terms of LC conditions, MS parameters and performance of method. The sensitivity for detection of levonorgestrel with ESI was 0.25 ng/mL which was lower than 1 ng/mL with APCI. Matrix effects were evaluated for levonorgestrel and canrenone (internal standard, IS) in human plasma, and the results showed that APCI source appeared to be slightly less liable to matrix effect than ESI source. With an overall consideration, ESI was chosen as a better ionization technique for rapid and sensitive quantification of levonorgestrel. The optimized LC–ESI–MS/MS method was validated for a linear range of 0.25–50 ng/mL with a correlation coefficient ≥0.99. The intra- and inter-batch precision and accuracy were within 11.72% and 6.58%, respectively. The application of this method was demonstrated by a bioequivalence study following a single oral administration of 1.5 mg levonorgestrel tablets in 21 Chinese healthy female volunteers.

1. Introduction

Levonorgestrel (Fig. 1A) is the most widely used synthetic female contraceptive hormone in pregnancy prevention in humans [1]. Racemic mixture was first synthesized in the 1950s, and the biologically active levorotary enantiomer is levonorgestrel. It has strong progestational and anti-ovulatory activities with no estrogenic effects [2]. Levonorgestrel is quickly and completely absorbed into systemic circulation after oral administration (bioavailability about 100%) [3]. As the use of oral contraceptives has increased globally, requirements of monitoring the pharmacokinetic behavior are gaining great importance [4].

Several methods have been reported for the determination of the concentration of levonorgestrel in animal [5] or human plasma [6–10]. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the most commonly used atmospheric pressure ionization sources for qualitative or quantitative liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis. Both ESI and APCI sources have been reported for determination of levonorgestrel. Solid phase extraction (SPE)–LC–MS/MS was used to determine levonorgestrel in human plasma with APCI as ionization source [7]. A large plasma volume (2 mL) was required to obtain a lower limit of quantification (LLOQ) by high condensation. An LC–MS/MS method was developed using ESI source with LLOQ of 0.25 ng/mL [8]. Although the runtime for each injection was only 2 min, serious matrix effects might occur when a large amount of human plasma samples was analyzed. Neither of these two experiments investigated matrix effects. Online SPE–LC–MS/MS with ESI source was applied for the simultaneous determination of six most frequently used synthetic progestins in human plasma samples, including levonorgestrel [9]. This method covered a quantification concentration range of 2–100 ng/mL, which was maladaptive for human pharmacokinetic study. A more sensitive LC–ESI–MS/MS method was developed for the quantification of levonorgestrel in human plasma by chemical derivatization using hydroxylamine [10]. Although the LLOQ was 0.1 ng/mL in this method, derivatization made the sample preparation complex and time consuming.

In this work, we evaluated two common ionization sources of LC–MS/MS system, ESI and APCI, in the analysis of levonorgestrel in human plasma with canrenone (Fig.1B) as internal standard (IS). Methods for both ESI and APCI sources were developed and optimized. Liquid-liquid extraction (LLE) with cyclohexane was employed. Linearity, quantitation limitation and the presence of matrix effects in both cases were discussed. LC–ESI–MS/MS method was chosen for...
a bioequivalence study in 21 Chinese healthy female volunteers following oral administration of levonorgestrel at the dose of 1.5 mg.

2. Experimental

2.1. Chemicals and reagents

Levonorgestrel reference standard (Batch No: 100076-201205) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Canrenone reference standard (Batch No: 101202-201001) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was from Merck (Darmstadt, Germany). Formic acid, sodium bicarbonate and cyclohexane of analytical grade were procured from Nanjing Chemical Reagents Co., Ltd. (Nanjing, China). Deionized water was purified using a Milli-Q Water Purification System (Merck Millipore, Darmstadt, Germany) after distilled twice and used throughout the experiment.

2.2. Preparation of stock and working solutions

The standard stock solutions of levonorgestrel and IS were prepared in methanol at the concentration of 1 mg/mL respectively and then stored at 4 °C. The working solutions of levonorgestrel used for calibration and quality control (QC) were prepared daily by further dilution of the standard stock solution in methanol:water (80:20, v/v). The IS working solution was prepared freshly by diluting the standard stock solution to 200 ng/mL.

2.3. LC–MS/MS instrumentation and conditions

The liquid chromatographic separation and mass spectrometric detection were achieved by employing the Nexera UHPLC system coupled to an LCMS-8040 tandem quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Labsolutions LCMS software (Shimadzu) was used to control the instruments and process the data. The Nexera UHPLC system used in the analysis consisted of a system controller...
The instrument was equipped with both ESI and APCI sources. Chromatographic separation was performed using a Shimadzu Shim-pack VP-OSD C18 column (150 mm x 2.0 mm, 4.6 µm) at a flow rate of 0.2 mL/min in ESI source, and a Shimadzu Shim-pack VP-OSD C18 column (150 mm x 4.6 mm, 4.6 µm) at a flow rate of 1.0 mL/min in APCI source. The isocratic mobile phase consisted of methanol and 0.01% formic acid (80:20, v/v). The mass spectrometer operating parameters were optimized as follows: interface voltage, 4.5 kV; nebulizer gas flow, 3 L/min; drying gas flow, 15 L/min; desolvation line (DL) temperature, 250 °C; heat block temperature, 400 °C in ESI source, 200 °C in APCI source. The APCI temperature was 350 °C. Other parameters were tuned automatically.

2.4. Sample preparation

All the samples were stored in the freezer at –20 °C, and allowed to thaw at ambient temperature prior to analysis. LLE was chosen to
prepare the samples. To a 500 µL aliquot of human plasma in a 10 mL glass tube, 10 µL of the IS working solution (200 ng/mL), 100 µL of the saturated sodium bicarbonate and 4 mL of cyclohexane were added. The mixture was vortex-mixed thoroughly for 3 min, and then centrifuged at 4000 rpm for 10 min. The upper organic layer was transferred to another 10 mL clean glass tube and evaporated to dryness at 40 °C under a steady stream of nitrogen. The residue was reconstituted in 150 µL mobile phase, and vortex-mixed for 1 min. After centrifuging at 14,000 rpm for 10 min, a 10 µL of the supernatant was injected into the LC–MS/MS system for analysis.

2.5. Calibration curve and QC samples

Calibration standards were prepared by spiking 0.5 mL blank human plasma with proper volume of levonorgestrel working solutions, producing levonorgestrel plasma concentrations of 0.25, 0.5, 1, 2, 5, 8, 10, 20, and 50 ng/mL. QC samples were prepared by spiking blank plasma with proper volume of the working solutions to produce a final concentration equivalent to 0.5 ng/mL (low level), 5 ng/mL (middle level) and 20 ng/mL (high level) of levonorgestrel. These samples with known amounts of levonorgestrel were extracted as described in the section “Sample preparation”. In each run, two blank samples (processed with or without the IS, respectively) were analyzed to confirm absence of interferences but not used to construct the calibration function. Calibration curves were constructed by determining the best fit of peak area ratios (peak area of analyte/peak area of IS), where X

![Representative chromatograms for levonorgestrel and the IS resulting from analysis of 5 ng/mL of levonorgestrel in human plasma with (A) ESI source and (B) APCI source.](image-url)

Table 2

Matrix effects for levonorgestrel and canrenone by ESI and APCI (n=5).

| Concentration (ng/mL) | ESI Matrix effect (%) | ESI RSD (%) | APCI Matrix effect (%) | APCI RSD (%) |
|----------------------|-----------------------|-------------|-----------------------|-------------|
| Levonorgestrel        |                       |             |                       |             |
| 0.5                  | 131.74                | 6.28        | ND                    | ND          |
| 5                    | 128.12                | 3.28        | 104.66                | 8.65        |
| 20                   | 125.13                | 4.27        | 99.17                 | 8.37        |
| Canrenone             |                       |             |                       |             |
| 0.4                  | 126.17                | 4.04        | 123.14                | 5.13        |

ND: not detected.

Table 3

The intra- and inter-batch precision and accuracy of the method for the determination of levonorgestrel.

| Conc. added (ng/mL) | Intra-batch (n=5) | Inter-batch (n=15) |
|---------------------|-------------------|--------------------|
|                     | Determined Conc. (ng/mL) | RSD (%) | Accuracy (%) | Determined Conc. (ng/mL) | RSD (%) | Accuracy (%) |
| 0.5                 | 0.51              | 11.72             | 101.77        | 0.50                 | 9.85     | 99.20       |
| 5                   | 4.67              | 5.06              | 93.42         | 4.75                 | 8.26     | 94.91       |
| 20                  | 20.08             | 7.71              | 100.42        | 19.51                | 6.48     | 97.54       |
3. Results and discussion

3.1. Choice of LC conditions

A Shimadzu Shim-pack VP-OSD C18 column (150 mm×2.0 mm, 4.6 µm) at a flow rate of 0.2 mL/min was used in ESI source. The chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials. With the method of orthogonal experimental design, three concentration levels of formic acid and ammonium acetate buffer in aqueous phase were investigated. Different ratios of methanol as organic phase were also considered. Based on these results, the adopted mobile phase is a mixture of methanol and 0.01% formic acid (80:20, v/v) for balancing the chromatographic separation and MS/MS sensitivity. This condition successfully separated IS and levonorgestrel at retention time of 3.4 and 4.0 min, respectively, which avoided the interference of ionization between them. In APCI source, abovementioned mobile phase compositions were investigated as well. Similar results were obtained, so the same mobile phase was used as ESI source. Since the column effluent flow-rate has to be considered an important factor for the sensitivity of LC–APCI–MS methods [11,12], flow rates of 0.2, 0.5 and 1.0 mL/min were investigated by two inner diameter sizes of Shimadzu Shim-pack VP-OSD C18 column (150 mm×2.0 mm and 150 mm×4.6 mm) respectively. The sensitivity with APCI source was observed to increase when the solvent flow rate increased. Therefore, a Shimadzu Shim-pack VP-OSD C18 column (150 mm×4.6 mm, 4.6 µm) at a flow rate of 1.0 mL/min was used in APCI source.

3.2. Optimization of MS parameters (ESI and APCI)

The working parameters of ESI and APCI ionization sources for detection of levonorgestrel and IS were optimized automatically by injecting 1 µL of the standard solutions (1 µg/mL) into different sources separately and the MS/MS spectra were recorded and compared. Both the positive and negative modes were investigated in two sources. The response of positive ions was stronger than that of negative ions no matter which source was used, which indicated that the positive mode was much more sensitive. Therefore, all detections were operated in a positive multiple-reaction monitoring (MRM) mode. The optimized results of product ions m/z were 245.10, 109.10, and 91.10, corresponding to collision energies of 19 V, 28 V and 48 V in priority. With ESI source, there was strong interference when precursor to product transition m/z 313.10→245.10 was used for detecting low concentration levonorgestrel in plasma. Therefore, m/z 313.10→109.10 was chosen for quantification of levonorgestrel with ESI source while m/z 313.10→245.10 was chosen with APCI source. The precursor to product transition m/z 341.10→107.10 for IS was used in both two sources as optimal result. There was no endogenous interference present at the retention time of 4.0 min for levonorgestrel and 3.4 min for IS.

3.3. Method performance

3.3.1. Specificity

Blank plasma used to assay the specificity of the method was obtained from six healthy individuals. Specificity was investigated by comparing the chromatograms of blank human plasma (Fig. 2) with those of spiked human plasma samples in which the concentration of levonorgestrel closed to the LLOQ (Fig. 3). In all the tested six blank human plasma chromatograms, no endogenous compounds peak was observed at the retention time of levonorgestrel and IS in both APCI and ESI sources.

3.3.2. Linearity and quantification limits

Levonorgestrel calibration standard solutions were analyzed by three replicate analyses by ESI and APCI, respectively. Calibration
curves were prepared on three different days. With ESI source, the calibration curves showed good linearity in the range of 0.25–50 ng/mL, while the range with APCI was just 1–50 ng/mL. Results of three representative calibration curves for LC–MS/MS determination of levonorgestrel are given in Table 1. The representative chromatograms for levonorgestrel and the IS resulting from analysis of 5 ng/mL of levonorgestrel in human plasma with ESI and APCI sources are shown in Fig. 4.

The LLOQ was assessed by repeatedly analyzing the spiked plasma samples in five replicates, in which the deviation was within ± 20% of the nominal concentration. The LLOQ of levonorgestrel was 0.25 ng/mL with ESI source while 1 ng/mL with APCI source.

3.3.3. Matrix effects

LC–MS/MS is a perfect strategy for biological sample analysis because of its precision, robustness, high sensitivity and selectivity. However, one limitation of LC–MS/MS that has been increasingly taken into consideration in recent years is the matrix effect [13–15]. Both ESI and APCI have been reported to suffer from this phenomenon [16]. To evaluate the matrix effects for the two sources, the peak areas of the levonorgestrel dissolved in blank sample (the final solution of blank plasma after being extracted and redissolved) were compared with those obtained by injection of standard solutions at the same concentration. The matrix effect of IS (4 ng/mL in plasma) was performed in the same way. The matrix effect values for levonorgestrel and IS with ESI and APCI sources are shown in Table 2. The blank human plasma used in this assay was obtained from different sources. The results indicated that APCI was less susceptible to matrix effect than ESI source. This finding is in agreement with previously reported [17,18].

3.4. Summary of comparison

Analytes in liquid stream are converted into gas phase ions in both ESI and APCI sources to adapt the mass spectrometer detection [19,20]. However, the way to produce ionized analyte is very different. In ESI, analyte is ionized in the liquid phase inside the electrically charged droplets, and then analyte ions in solution are liberated from the liquid phase into the gas phase [21]. In APCI, the neutral analyte is transferred into the gas phase by vaporizing the liquid in a heated gas stream, and the gas phase analyte is chemically ionized in a separate step [22]. So mobile phase composition, specificity, linearity, quantification limits and matrix effects were taken into consideration in this comparison study. Both ESI and APCI sources in this experiment have no endogenous substances and metabolites interfering with the assay of levonorgestrel and IS. The LC–ESI–MS/MS/MS method was proved to be more sensitive for measuring levonorgestrel in human plasma with an LLOQ at 0.25 ng/mL, while APCI at 1 ng/mL. ESI showed a small but constant matrix effect in different concentrations of levonorgestrel in plasma (0.5, 5 and 20 ng/mL), while APCI showed scarcely any matrix effect. Since levonorgestrel has low blood level after oral administration, ESI source was employed in human pharmacokinetic study.

3.5. Robustness and ruggedness

Robustness and ruggedness were determined to evaluate the influence of small but deliberate variation in the chromatographic conditions. The robustness of the method was evaluated in flow rate, mobile phase composition and injection volume. In method development, it was found that slight changes in flow rate ( ± 0.01 mL/min) and mobile phase composition (ratio of methanol in mobile phase ± 1%) had no significant influence on peak area value of levonorgestrel and IS. Injection volume had almost no effect on response of analyte and IS, either. Ruggedness was investigated when the instrument was changed to the Finnigan™ TSQ Quantum Discovery MAX™ LC–MS/MS system. Variations of chromatographic behavior and MS response were acceptable. Because of this, it is feasible to perform method validation and pharmacokinetics study in the Finnigan™ TSQ Quantum Discovery MAX™ LC–MS/MS system which equipped with ESI source.

3.6. Validation of the LC–ESI–MS/MS method

The LC–ESI–MS/MS method was validated following the United States Food and Drug Administration bioanalytical method validation guidance [23].

Specificity, linearity and matrix effect had been investigated in the previous comparison study.

The precision and accuracy of intra-batch were determined by analyzing five sets of spiked plasma samples of levonorgestrel at each QC level (0.5, 5 and 20 ng/mL) in a batch. The precision and accuracy of inter-batch were determined by analyzing five sets of spiked plasma samples of levonorgestrel at each QC level on three consecutive batches. The concentration of each sample was calculated by the calibration curve prepared and analyzed in the same day. Results are shown in Table 3. The intra- and inter-batch precision was in the range of 5.06%–11.72% and 6.48%–9.95%, respectively. The intra- and inter-batch accuracy had been investigated in the previous comparison study.

The absolute extraction recovery of levonorgestrel was assessed by comparing the analyte and IS peak area ratios in processed samples to blank plasma extracts spiked with parallel concentration solutions. This procedure was repeated (n=5) at each QC level. The extraction recovery determined for levonorgestrel was consistent, precise and reproducible. The mean recoveries of levonorgestrel at the three concentration levels were 80.21%, 77.37% and 82.95%, respectively, whereas the RSDs were 6.21%, 2.80% and 13.73%, respectively. The mean absolute extraction recovery of the IS was 72.74% ± 11.44% at the concentration used in the assay (4 ng/mL).

The stability of levonorgestrel in human plasma under different conditions was assessed by simulating conditions that might occur during the experiment. Table 4 summarizes the results of stability experiments of levonorgestrel. The results showed the reliable stability behavior of levonorgestrel under the tested conditions. The stability of levonorgestrel and IS in stock solutions were also examined and no obvious reduction was found during the assay.

3.7. Application in pharmacokinetics study

The validated method was successfully applied to a pharmacokinetic study following oral administration of levonorgestrel at the dose of 1.5 mg. A total of 21 Chinese healthy female volunteers took part in the pharmacokinetic study (Ethical Committee License Number: YW20140321-03). Blood samples (4 mL) were collected before intake and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24, 48, and 72 h after oral administration. The samples were put into lithium heparin tubes and were immediately centrifuged at 3000 rpm for 10 min. The plasma obtained was frozen at −20 °C in coded polypropylene tubes until analysis. The pharmacokinetic parameters were calculated using the Drug and Statistics (DAS) version 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). All the mean plasma concentration-time profiles of 21 volunteers are represented in Fig. 5. Pharmacokinetic parameters of the test orally tablet and reference tablet are listed in Table 5. The test tablets were found to be bioequivalence to the reference ones.

3.8. Incurred sample reanalysis (ISR)

The quality of the bioanalytical assay was assessed by ISR. 24 samples were chosen for ISR per period, and 48 in whole pharmacokinetics study. All samples met the criteria of being close to the maximum concentration or near the LLOQ. Those selected samples
had percentage difference from ~17.66% to 19.77%, as well as 11.80% for mean deviation. The results authenticated the reproducibility of the proposed method.

4. Conclusion

Two ionization techniques, ESI and APCI for LC–MS/MS system were compared for quantification of levonorgestrel. The LLOQ, linearity, and matrix effects were evaluated for levonorgestrel in human plasma. The results show that APCI source was less affected by the ion suppression produced by sample matrix components, but the LLOQ of levonorgestrel for the ESI source was lower than that for the APCI source. This rule can be widely applied to quantification of endogenous and exogenous progestogens.

The results show that the ESI source is more attractive because high sensitivity was obtained. An LC–ESI–MS/MS method was validated for the quantification of levonorgestrel in human plasma. The method was rapid, selective and highly sensitive with an LLOQ at 0.25 ng/mL for levonorgestrel. The analysis time is only 5.0 min for each run. The simple preparation, rapid separation, and good reproducibility of analysis are the most outstanding characteristics of this method. The method has been successfully applied to evaluate the pharmacokinetics of levonorgestrel following oral administration at the dose of 1.5 mg to healthy Chinese female volunteers.

Acknowledgments

Thanks for all co-workers in Nanjing First Hospital and China Pharmaceutical University.

References

[1] F.Z. Stanczyk, J.P. Happegood, S. Winer, et al., Progestogens used in postmenopausal hormone therapy: differences in their pharmacological properties, intracellular actions, and clinical effects, Endocr. Rev. 34 (2012) 171–208.

[2] K. Kook, H. Gabelnick, G. Duncan, Pharmacokinetics of levonorgestrel 0.75 mg tablets, Contraception 66 (2002) 73–78.

[3] W.X. Ding, X.R. Qi, Q. Fu, et al., Pharmacokinetics and pharmacodynamics of sterylglucoside-modified liposomes for levonorgestrel delivery via nasal route, Drug Deliv. 14 (2007) 101–104.

[4] R. Sitruk-Ware, A. Nath, Characteristics and metabolic effects of estrogen and progestins contained in oral contraceptive pills, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 13–24.

[5] S. Ananthula, D.R. Janagam, S. Jamalapuram, et al., Development and validation of sensitive LC/MS/MS method for quantitative bioanalysis of levonorgestrel in rat plasma and application to pharmacokinetics study, J. Chromatogr. B 1003 (2015) 47–53.

[6] A. Gandhi, S. Guttikar, P. Trivedi, High-sensitivity simultaneous liquid chromatography-tandem mass spectrometry assay of ethinyl estradiol and levonorgestrel in human plasma, J. Pharm. Anal. 5 (2015) 316–326.

[7] C. Hubert, B. Strel, R. Sibenaler, et al., Proceedings of the 31st International Symposium on High Performance Liquid Phase Separations and Related Techniques, Ghent, Belgium, 2007.

[8] L.Z. Zhao, G.P. Zhong, H.C. Bi, et al., Determination of levonorgestrel in human plasma by liquid chromatography-tandem mass spectrometry method: application to a bioequivalence study of two formulations in healthy volunteers, Biomed. Chromatogr. 22 (2008) 519–526.

[9] C. Moser, D. Zoderer, G. Luef, et al., Simultaneous online SPE-LC/MS/MS quantification of six widely used synthetic progestins in human plasma, Anal. Bioanal. Chem. 403 (2012) 961–972.

[10] V.P. Kumar, A. Saxena, A. Pawar, et al., A rapid and sensitive UPLC-ESI-MS/MS method for determination of levonorgestrel by chemical derivatization in human plasma and its application to pharmacokinetic study, J. Anal. Bioanal. Tech. 86 (2014) 003.

[11] A. Asperger, J. Efer, T. Koal, et al., On the signal response of various pesticides in electrospray and atmospheric pressure chemical ionization depending on the flow-rate of eluent applied in liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 937 (2001) 65–72.

[12] T.J. Kauppila, A.P. Bruins, R. Kostiaisen, Effect of the solvent flow rate on the ionization efficiency in atmospheric pressure photoionization-mass spectrometry, J. Am. Soc. Mass Spectr. 16 (2005) 1399–1407.

[13] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (2003) 3019–3030.

[14] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, et al., Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses, J. Chromatogr. B 852 (2007) 22–34.

[15] M. Gros, M. Petrović, D. Barcelo, Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters, Talanta 70 (2006) 678–690.

[16] T. Sangster, M. Spence, P. Sinclair, et al., Unexpected observation of ion suppression in a liquid chromatography/atmospheric pressure chemical ionization mass spectrometric bioanalytical method, Rapid Commun. Mass Spectrom. 18 (2004) 1361–1364.

[17] B.K. Matuszewski, Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis, J. Chromatogr. B 830 (2006) 293–300.

[18] S. Souverain, S. Rudaz, J.L. Veuthey, Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures, J. Chromatogr. A 1068 (2004) 61–66.

[19] D.J. Weston, Ambient ionization mass spectrometry: current understanding of mechanistic theory; analytical performance and application areas, Analyst 35 (2010) 661–668.

[20] P.M.L. Sinues, E. Criado, G. Vidal, Mechanistic study on the ionization of trace electrospray and atmospheric pressure chemical ionization mass spectrometry, Anal. Chem. 65 (1993) 972A–986A.

[21] J. Sunner, G. Nicol, P. Kebarle, Factors determining relative sensitivity of analytes with o- and n-line and on-line extraction procedures, J. Chromatogr. A 1058 (2004) 986A–990.

[22] R. Wang et al.

Journal of Pharmaceutical Analysis 6 (2016) 356–362

[23] R. Wang et al.

Journal of Pharmaceutical Analysis 6 (2016) 356–362