Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 853–859

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of an improved high-throughput method for the determination of anastrozole in human plasma by LC–MS/MS and atmospheric pressure chemical ionization

Constantinos Apostolou, Yannis Dotsikas, Constantinos Kousoulos, Yannis L. Loukas*

Laboratory of Pharmaceutical Analysis and Bioequivalence Services (GLP Compliant), Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimioupoli Zografou GR, 157 71 Athens, Greece

ARTICLE INFO

Article history:
Received 2 March 2008
Received in revised form 5 June 2008
Accepted 6 June 2008
Available online 18 June 2008

Keywords:
Anastrozole
APCI
LC–MS/MS
96-Well
Bioequivalence

ABSTRACT

In the present study, an automated, 96-well format LC–MS/MS method for the determination of anastrozole in human plasma was developed and fully validated. Within method development procedure, atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were compared in terms of sensitivity and specificity, with the former proven to be more appropriate and thus being chosen for analyte ionization. In addition, the effect of declustering potential (DP) and collision energy (CE) in sensitivity was, as well, studied and compared between APCI and ESI source employment. Samples were treated with an acetonitrile (ACN) protein precipitation step followed by liquid–liquid extraction (LLE) with methyl *t*-butyl ether (MTBE) as the organic solvent, using omeprazole as the internal standard (IS). The statistical evaluation for the APCI protocol revealed excellent linearity, accuracy and precision values for the range of concentrations 0.100–100 ng/mL. The method proposed involves the lowest plasma volume so far reported (190 μL), as well as the shortest run time (1.6 min) and along with the employment of two robotic liquid handling systems enabled the rapid and reliable determination of anastrozole in a bioequivalence study (>1000 plasma samples) after *per os* administration of 1 mg tablet within a 4-day period of time.

1. Introduction

Anastrozole [2,2′-[5-1*H*-1,2,4-triazole-1-y-methyl)-1,3-phenylene]bis(2-methyl propiononitrile)], is a potent, selective, non-steroidal triazole aromatase inhibitor therapeutically used to treat breast cancer in post-menopausal women [1]. It inhibits aromatase activity by competitively binding to the heme of the cytochrome P450 subunit of the enzyme [2].

Anastrozole determination in biological fluids is a challenging task due to the relatively low recommended therapeutic dose (1 mg). A limited number of analytical methods have been so far proposed for anastrozole determination in human plasma, among which a few employing capillary gas chromatographic techniques with Ni^{63} electron capture detection [3–5] and only one LC–MS/MS method with photospray ion source (APPI) [6]. However, the specific ionization source is rarely found in analytical laboratories compared to electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources.

APCI and ESI are today considered to be complementary ionization techniques, as a result of which, performance comparison at the stage of analytical method development between the two ion sources has become increasingly frequent over the last few years [7–9]. Both APCI and ESI are atmospheric pressure ionization sources causing preferentially the formation of de-protonated or protonated parent ions without fragmentation. However, they are based on fundamentally different ion formation mechanisms, a fact which significantly differentiates several of their attributes [10–13].

In the present study, an automated, high-throughput 96-well format method was developed and fully validated. Within the stage of method development, the performance of APCI LC–MS/MS was compared to ESI LC–MS/MS in terms of sensitivity and specificity. Significant signal enhancement was observed when APCI was employed for both anastrozole and internal standard (IS) determination. In addition, severe signal alterations appeared when ESI was used, due to matrix effect, while APCI was proven to be unaffected by the presence of matrix components. Furthermore, declustering potential (DP) and collision energy (CE) effect in signal intensity was studied for both sources. Method sensitivity enhancement was further achieved by employing an ACN protein precipitation step

* Corresponding author. Tel.: +30 210 7274224; fax: +30 210 7274224.
E-mail address: loukas@pharm.uoa.gr (Y.L. Loukas).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.06.006

prior to liquid–liquid extraction (LLE) with methyl *t*-butyl ether (MTBE). The developed and fully validated method was applied to the fast and reliable determination of anastrozole in a bioequivalence study after *per os* administration of a 1-mg tablet to 24 healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Anastrozole was donated from Lamda Pharmaceuticals (Athens, Greece) while omeprazole was obtained from Cipla (Mumbai, India). Acetonitrile (HPLC grade) and ammonium acetate (analysis grade) were purchased from Chemilab (Athens, Greece). MeOH (HPLC grade) was obtained from Neohimiki (Athens, Greece) while MTBE (HPLC grade) from Techline (Athens, Greece). All aqueous solutions and buffers were prepared using de-ionized and doubly distilled water (resistivity > 18 MΩ) from a Millipore Milli-Q Plus System (Malva, Athens, Greece). Pooled human control plasma was kindly donated from Ippokrateio hospital (Athens, Greece).

2.2. Instrumentation

All liquid transfers, including buffer, IS addition, as well as plasma samples transferring from 2 mL Eppendorf microfuge tubes (Lab Supplies, Athens, Greece) into 2.2 mL square 96 deep-well plates (Sigma–Aldrich, Athens, Greece) were performed by a PerkinElmer Multiprobe II HT-EX workstation (PerkinElmer, Downers Grove, IL, USA) equipped with an 8-tip robotic arm and controlled by WinPrep Software. 200 μL conductive disposable tipboxes were purchased from E&K Scientific Products (Cambell, CA, USA). A tipchute, reagent troughs and a tip flush/wash station were purchased from PerkinElmer. ACN and MTBE addition, supernatant organic layer transferring after extraction into a new 2.2 mL 96 deep-well plate as well as sample reconstitution after evaporation were performed by a Tomtec Quadra 96 model 320 robotic liquid handling system equipped with a 96-tip pipetting head (Bidservice, NJ, USA). An Eppendorf 5810 R (Bacakos, Athens, Greece) centrifuge that could accommodate 96-well plates as well as Eppendorf microfuge tubes was also utilized during sample preparation. Evaporation was performed into a Zymark TurboVap 96-well format plate evaporator (Malva, Athens, Greece) by nitrogen application, produced by an Agilent Nitrogen Generator (Duratec, Hockenheim, Germany), that receives air from a SF4 Air Compressor (Atlas Copco, Athens, Greece). Eppendorf deepwell mats for covering the 96-well plates were obtained from Sigma–Aldrich. Two 96-well plate vortex-mixers (MS1 Minishaker) were purchased from Metrolab (Athens, Greece). The CTC PAL autosampler (Hellamco, Athens, Greece) could accommodate six 96 deep-well plates kept before analysis at a fixed temperature inside each autosampler drawer. The HPLC system included one Agilent 1100 series binary pump, a degasser as well as a column oven/cooler (Hellamco, Athens, Greece). Finally, a PE Sciex API 3000 triple quadrupole mass spectrometer (Biosolutions, Athens, Greece) interfaced with the HPLC via a heated nebulizer (APCI) or an ESI source was used for the mass analysis and detection, operating under Analyst 1.4.2 software.

2.3. Chromatographic conditions

The mobile phase consisted of 90% organic mixture acetonitrile/methanol 90/10 and 10% 10 mM ammonium acetate (v/v), adjusted to pH 3.5 with acetic acid. A flow rate of 0.6 mL/min was used for mobile phase isocratic delivery on a YMC cyano (Schermbeck, Germany) analytical column (50 mm × 4.0 mm i.d). The pressure of the system during the analysis was ∼350 psi. The column was maintained at ambient temperature (∼23 °C), while the autosampler temperature was set at 10 °C. The injection volume was 30 μL and the total run time was set for 1.6 min.

2.4. Mass spectrometric conditions

A heated nebulizer (APCI) and a turbo ionspray (ESI) source were interfaced with a PE Sciex API 3000 triple quadrupole mass spectrometer for mass analysis and detection. Source temperature was optimized at 400 °C, while corona discharge needle was set at 2 mA. Auxiliary and curtain gas were set at 3 and 6 a.u. (arbitrary units), respectively, while collision gas was set at 12 a.u. The curtain gas was set at 10 a.u., the DP at 41 V for anastrozole, and 15 V for omeprazole. The nebulizer gas (GS1) was set at 5 a.u., while the turbo ionspray gas (GS2) at 7 L/min. The collision gas setting was optimized at 10 a.u., the CE at 31 V for anastrozole and 15 V for omeprazole. The analytes were detected by monitoring the precursor → product ion transition using multiple reaction monitoring (MRM) scan mode. The turbo ionspray source temperature was optimized at 400 °C. Nebulizer, curtain and collision gas were optimized at 5, 10 and 10 a.u., respectively. Finally, ionspray voltage, collision energy, DP, focusing potential (FP) and cell exit potential were set at 4500, 27, 81, 170 and 6 V, respectively. The MRM was performed with both ion sources at m/z 294.3 → 225.4 and m/z 346.2 → 198.3 for anastrozole and omeprazole, respectively.

2.5. Standards and quality control/method validation (QC/MV) samples preparation

Separate stock solutions of anastrozole and omeprazole {(SA_1) and (IS_1)} were prepared in MeOH at concentration of 100 μg/mL each. A quality control/method validation stock solution (100 μg/mL) was prepared from separate weighing of anastrozole. Working solutions of 2.00×10^3, 1.00×10^3, 400, 200, 100, 40.0, 20.0, 10.0, 4.00 and 2.00 ng/mL for anastrozole were prepared by diluting SA_1 with MeOH/H_2O 50/50 (v/v). Dilutions were used to prepare four levels of QC working solutions, 1.50×10^3, 150.0, 6.00 and 2.00 ng/mL. A diluted IS working solution (800 ng/mL, IS_2) was prepared daily by diluting the IS stock solutions in MeOH. All stock and working solutions were stored at 4 °C. Ten calibration standards were prepared by spiking 100 μL of each working solution to 1900 μL of blank human plasma (20-fold dilution). A blank sample (matrix sample processed without IS), a zero sample (matrix sample processed with IS) completed the calibration curve covering the expected range of concentrations to be quantified. Final standard concentrations were 100, 50.0, 20.0, 10.0, 5.00, 2.00, 1.00, 0.500, 0.200, and 0.100 ng/mL. The following concentration levels of QC/MV samples were prepared: MV_L (0.100 ng/mL), MV_1/QC_1 (0.300 ng/mL), MV_2/QC_2 (7.50 ng/mL) and MV_3/QC_3 (75.0 ng/mL). Standards were placed and stored into properly labeled Eppendorf tubes at −30 °C.

2.6. Sample preparation

After thawing at room temperature and vortex mixing, plasma samples were centrifuged for 5 min at 4 °C. Multiprobe transferred 190 μL of each calibrator, QC and subject sample into the appropriate wells of a 96-well plate, followed by 50 μL of IS_2 solution and plates were vortex mixed for 5 min. Next, 150 μL of ACN were added to all wells and the plates was vortexed for another 10 min so as to achieve satisfying protein precipitation. LLE was performed by the addition of 1100 μL of MTBE into all 96 wells of each plate. The plates were covered with a mat, vortex mixed for 20 min, centrifuged for 15 min at 3500 rpm and 4 °C and then frozen for 30 min at −30 °C. After careful removal of the mats, 900 μL of the super-

natant organic layer, were transferred from the original sample plates, into the respective positions of new 2.2 mL 96 deep-well plates and the new plates with the organic extracts were placed into nitrogen evaporator for 20 min. The resulting dry residues were reconstituted by the addition of 130 μL of mobile phase and the plates after vortex mixing for 5 min were placed into the autosampler for injection.

3. Results and discussion

3.1. Mass spectrometric optimization

Mass spectrometric parameters were optimized for both sources by infusing a 200-ng/mL standard solution of anastrozole and the IS in mobile phase at 30 μL/min, via an external syringe pump (Harvard 11 plus) directly connected to the mass spectrometer. APCI (+) and ESI (+) full-scan spectra of both compounds are presented in Fig. 1A and B, respectively. The two spectra were similar, showing an abundant $[M+H]^+$ peak at *m*/*z* 294.3. However, anastrozole precursor ion peak intensity in APCI spectrum appears to be almost 10 times higher than the equivalent of ESI.

Both APCI and ESI optimization procedure involved CE, DP, focusing potential, entrance potential (EP) and source temperature (TEM) adjustment. APCI optimization also included auxiliary gas (AUX), curtain gas (CUR), collision gas (CAD) and nebulizer current (NC) adjustment, while for ESI, nebulizer gas (NEB), curtain gas, collision gas, and ion spray voltage were adjusted to the optimal value.

Product ion selection and parameters optimization was performed for the three product ions of highest intensity. Fig. 2 shows the final anastrozole product ion scan, with major peaks at *m*/*z* 225.4 for both sources. Another common product ion for both spectra is found at *m*/*z* 115.2 while the third most intense peak for APCI and ESI appears at *m*/*z* 142.3 and 168.2, respectively.

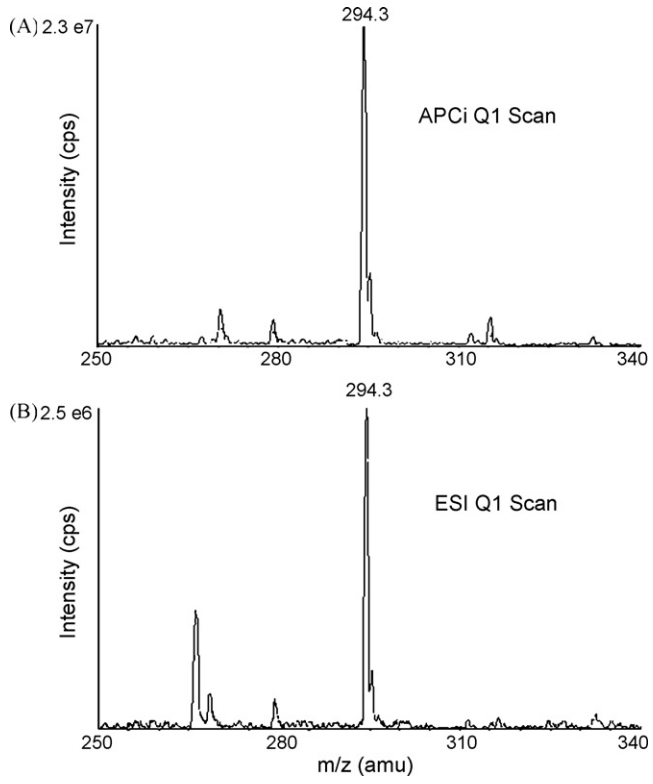

Fig. 1. Anastrozole APCI (A) and ESI (B) full mass scan.

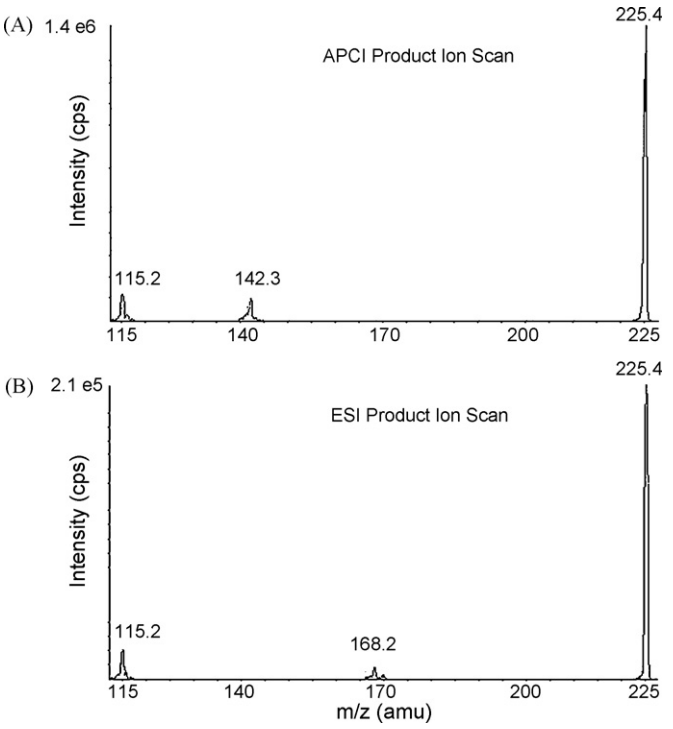

Fig. 2. Anastrozole APCI (A) and ESI (B) product ion scan.

Sensitivity comparison between methods employing APCI and ESI source was performed by preparing samples at four different anastrozole concentrations (0.100, 0.300, 7.50 and 75.0 ng/mL) in triplicate ($n = 3$), following the final, optimized sample preparation protocol. Samples were analyzed with both sources and results were compared in terms of mean peak area and mean peak signal to noise (S/N) ratio for each concentration level. Results presented in Table 1 reveal a 10-fold higher sensitivity of APCI compared to ESI for anastrozole determination in human plasma samples. APCI source was proven to result in threefold higher sensitivity of omeprazole determination as well compared to ESI (data not shown).

Sensitivity differentiation between ion sources is directly connected to molecular structure. Molecular weight, polarity and the existence of groups susceptible to ionization are the most important factors that define the compatibility of a molecule with a specific ion source. In general terms, ESI is a more effective ionization method for compounds of higher polarity than APCI is, although 'higher' is a very subjective term and there is a range of compounds for which both techniques are applicable. APCI is employed when molecules of low-medium polarity are to be determined, as long as a group capable of ionization is present, while ESI source is generally compatible with compounds of medium-high polarity [14]. Anastrozole and omeprazole are both compounds with relatively low molecular weight and medium polarity. Therefore, the differentiation of APCI vs. ESI sensitivity (comparison of peak areas by preparing plasma samples at four concentration levels) for anastrozole (10-fold) compared to omeprazole (threefold) is obviously attributed to the disparity in molecular polarity between the two compounds.

3.2. CE and DP effect in APCI and ESI sensitivity

CE and DP are two of the most important parameters for source optimization, highly affecting sensitivity. Their influence on APCI and ESI sensitivity for anastrozole detection was evaluated by

Table 1
APCI–ESI peak area and S/N ratio mean values for sensitivity comparison

(n = 3)	MV_L (0.100 ng/mL)	MV_1 (0.300 ng/mL)	MV_2 (7.50 ng/mL)	MV_3 (75.0 ng/mL)
Area (cps)				
APCI	6088	12,033	103,367	1,040,667
ESI	601	1,243	10,047	92,267
Signal to noise (S/N) ratio				
APCI	14	28	197	1,540
ESI	1.4	6.7	26	341

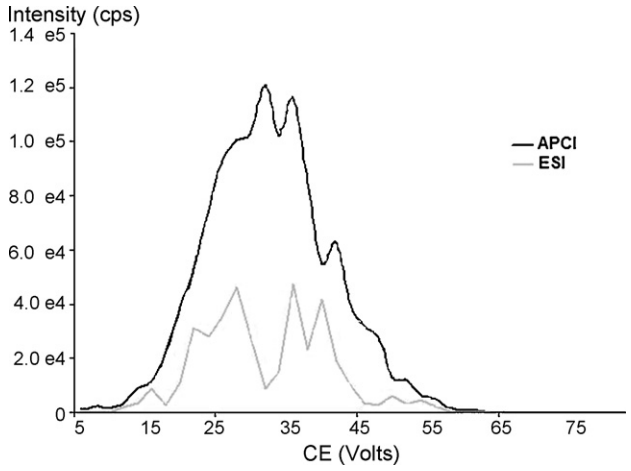

Fig. 3. Collision energy effect in product ion intensity.

studying the intensity of the most abundant product ion (m/z 225.4) when the values of these parameters changed. Several CE values were tested, ranging from 5 to 80 V, while for DP the testing range was 1–101 V. CE value variation highly affected sensitivity for both sources with optimum values lying within the narrow range of 25–40 V. A similar form appeared in DP effect evaluation for APCI with maximum intensities for DP values between 30 and 55 V with a peak at 41 V. On the contrary, when ESI was employed, DP variation did not produce a similar peak shaped graph and therefore indicating a less significant influence on ESI sensitivity for anastrozole (Figs. 3 and 4). From the data presented above, it is obvious that a cautious adjustment of DP and CE values is indispensable, especially when analytes in low concentrations are to be determined.

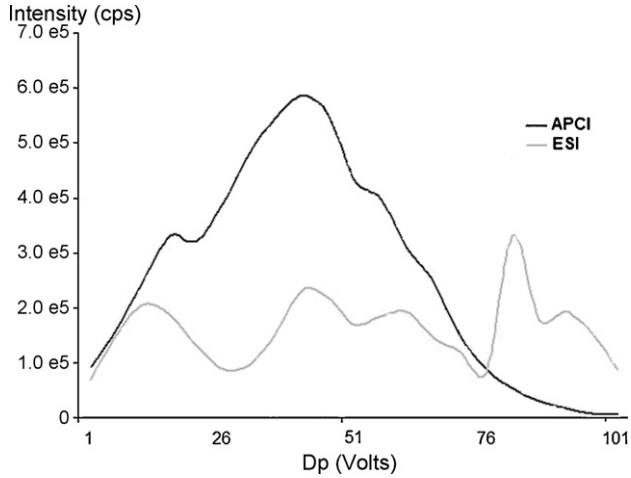

Fig. 4. Declustering potential effect in product ion intensity.

3.3. Sample preparation optimization

All analyses for sample preparation optimization were performed with APCI as the chosen ion source. The employment of Multiprobe and Tomtec robotic liquid handling workstations for all liquid transfer steps significantly reduced total time of sample preparation, while increasing at the same time the degree of procedure automation. Several organic solvents were evaluated for anastrozole and IS LLE extraction from plasma samples, including ethyl acetate, MTBE, as well as mixtures of diethyl ether and dichloromethane. Highest sensitivity, accuracy and precision were obtained when extraction was performed with MTBE. Further sensitivity increase was achieved by the employment of a protein precipitation step with ACN prior to MTBE addition. ACN volume which resulted in the highest sensitivity was equal to 190 μL, which was a 1:1 ratio to plasma volume. Samples in three anastrozole concentration levels (0.300, 7.50 and 75.0 ng/mL) were prepared in triplicate without ACN addition, as well as with the addition of 100, 190 and 250 μL ACN. Compared to LLE without an ACN protein precipitation step, anastrozole and omeprazole peak areas increased up to 87.5 and 106.8%, respectively when a protein precipitation step was included.

3.4. Chromatographic conditions optimization

Analytical columns tested were C_{18}, cyano and silica, with cyano producing the best results, while ACN, MeOH and different proportions between them were evaluated for mobile phase organic solvent. Ammonium acetate and formic acid were tested as buffer solutions with pH values ranging from 3.5 to 5.5. Ammonium acetate produced the sharpest peaks, while pH 4.5 significantly increased sensitivity. Finally, organic solvent percentage in mobile phase was optimized within the range of 70–95% with 90% being the percentage of choice, since it resulted in the highest anastrozole peak area. Representative MRM LC–MS/MS chromatograms are shown in Fig. 5. As far as the chromatographic profile of the method is concerned, the retention times were about 0.88 min for both compounds with a total run time of only 1.6 min, the shortest so far reported for anastrozole analysis.

3.5. Matrix effect comparison

Matrix effect is the signal suppression or enhancement caused mainly by the existence of remaining polar matrix components in the samples analyzed. Several approaches have been adopted to explain matrix effect in ESI [15–17] most of which attribute it to ionization competition between analytes and polar plasma components eluted from column. APCI is considered to be less susceptible to matrix effect, mainly because of the fact that contrary to ESI, ionization takes place in the gas phase [18–21].

In the present study, matrix effect for the whole chromatographic run was investigated for both APCI and ESI sources by the post-column infusion protocol [22,23]. Blank sample extracts were injected in the LC–MS/MS system by the simultaneous post-

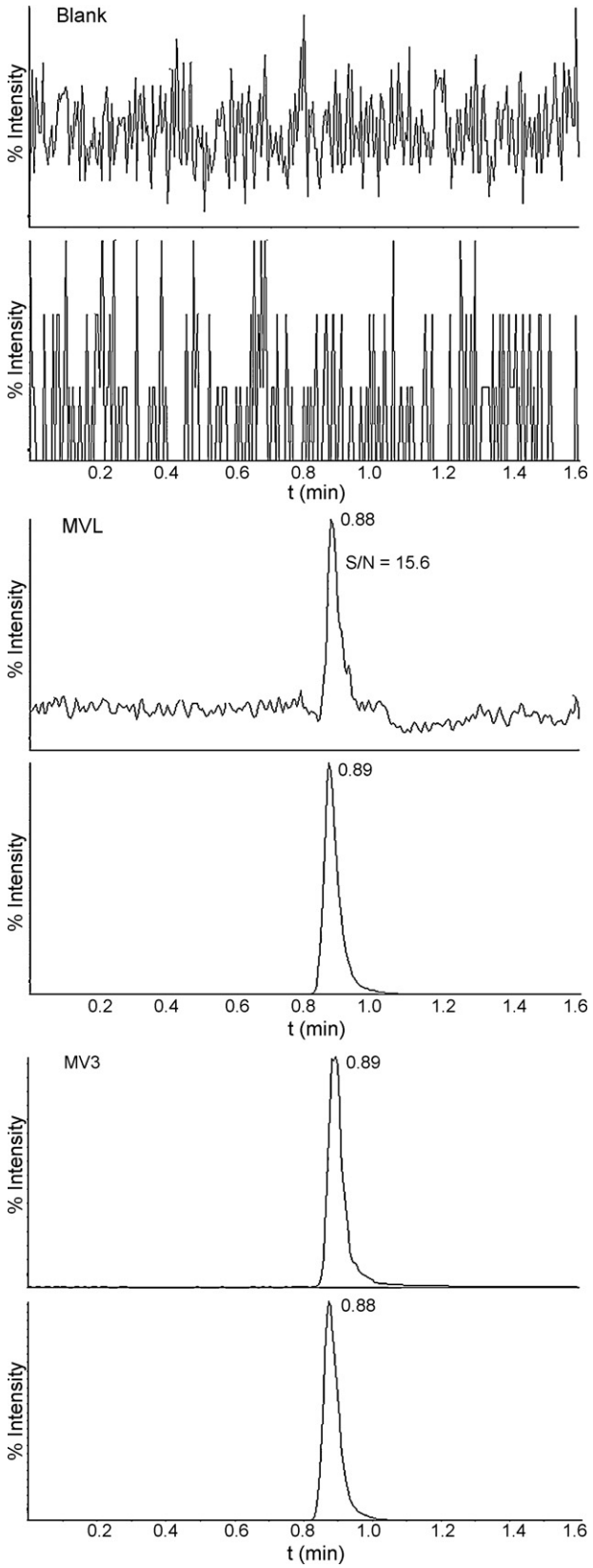

Fig. 5. Representative MRM chromatogram of anastrozole (top) and IS obtained from a blank, MV_L and a MV_3 sample.

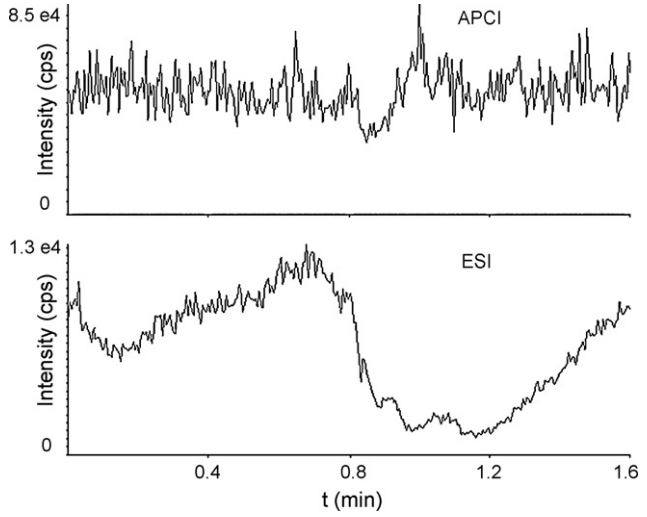

Fig. 6. Anastrozole matrix effect chromatographic profile for both ionization sources.

column infusion of a mixture of analyte and IS at 100 ng/mL in mobile phase via the Harvard syringe pump. The flow rate was set at 20 μL/min, while the syringe pump was connected in parallel via a PEEK tee.

As for the APCI source, mass spectrometric signal for both anastrozole and omeprazole remained practically unaffected by the post-column infusion of blank extract as can be seen in Figs. 6 and 7. On the contrary, when the same procedure was repeated with the ESI source, severe mass spectrometric signal alterations were observed. These remarks are in accordance with the previous theoretical considerations.

Matrix effect was determined quantitatively by comparing analyte peak area counts from plasma samples fortified with analyte at three concentration levels (0.300, 7.50 and 75.0 ng/mL) as well as IS post-extraction, to samples from neat solutions at the same concentrations for analyte and IS. Numerical values (%) for each concentration level were calculated by dividing the area of plasma extracted sample spiked with analyte and IS, by the area of the respective neat solution and were equal to 86.3, 82.7 and 84.9% ($n = 3$) respectively, while for the IS it was equal to 88.7% ($n = 9$).

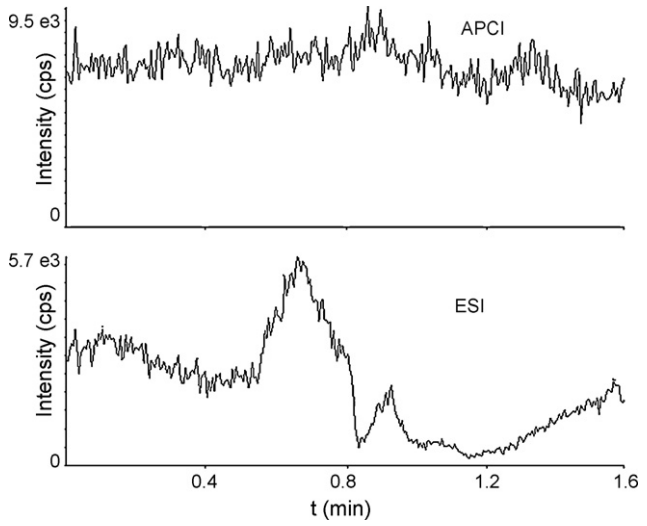

Fig. 7. Omeprazole matrix effect chromatographic profile for both ionization sources.

Table 2
Intra- and inter-assay accuracy and precision results

MV sample	% Intra-run accuracy[a]	% Inter-run accuracy[b]	Intra-run precision[c] (%CV)	Inter-run precision[b] (%CV)
MV_L (0.100 ng/mL)	102	104	13	9.7
MV_1 (0.300 ng/mL)	101	102	11	4.8
MV_2 (7.50 ng/mL)	99.6	100	7.9	5.0
MV_3 (75.0 ng/mL)	104	104	7.4	5.8

[a] $n = 6$, expressed as (mean calculated concentration)/(nominal concentration) × 100.
[b] Values obtained from all 5 runs ($n = 30$).
[c] $n = 5$.

3.6. Standard curve and method validation

Initially, a homoscedasticity test was performed by plotting residuals vs. concentration and by applying *F*-test. The results evidenced heteroscedastic situation and therefore the suitable weighting factor w_i should be selected. The following weighting factors were available via Analyst: $1/x$, $1/x^2$, $1/y$, $1/y^2$. The best weighting factor is chosen according to a percentage relative error (%RE), which compares the regressed concentration computed from the regression equation obtained for each w_i, with the nominal standard concentration. The weighting factor $1/x^2$ produced the least sum for this data set providing the most adequate approximation of variance. Thus, the $1/x^2$-weighting factor was chosen.

The limit of quantitation (LOQ) of the current analytical method was set at 0.100 ng/mL with a calibration curve containing 10 non-zero standards ranging from 0.100 to 100 ng/mL. Five analytical runs were prepared in order for full validation to be performed according to US Food and Drug Administration (FDA) bioanalytical method validation guidance (CDER, 2001) [24]. Calibration curves of the 5 analytical runs were linear in the specific range with the regression coefficients (R^2) being greater than 0.994. Average linear slope was 0.020 ($S_b = 0.00008$) and average intercept was 0.005 ($S_a = 0.00300$). The result of the proportionality test was also positive; the *t*-test experimental value of 1.806 was smaller than the theoretical value of 2.228 (5%, two-sided). As a result, the concentration data produced by the 96-well LLE procedure applied in this method was satisfactory for anastrozole standard samples.

However, before applying a statistical hypothesis test to the regression line coefficients it is essential to check whether "lack of fit" exists. This test is based on the analysis of the variance of the residuals from the regression line. Of primary interest is the *P*-value obtained from ANOVA. Small values of P (<0.05) indicate significant lack of fit at the 5% significance level. In this test a P value of 0.9978 indicated that there was no significant lack of fit and that the current method was capable of producing satisfactory concentration data for anastrozole standard samples.

Data for accuracy and both intra- and inter-run precision (expressed as CV%) are presented in Table 2. Extraction recovery results of the final method were 70.8, 64.9 and 72.2% for the three concentration levels tested (0.300, 7.50 and 75.0 ng/mL), respectively, while for omeprazole, recovery was found equal to 69.3%.

Finally, autosampler stability, freeze and thaw stability, short term room temperature stability, long-term stability as well as working solutions and master stock solutions stability were assessed as part of the method validation protocol with all results lying within the acceptable limits (data not shown).

3.7. Application to a bioequivalence study

The present method was applied to the analysis of plasma samples obtained from 24 healthy male volunteers after the administration of 1 mg tablet of anastrozole, as part of a bioequivalence study. The pharmacokinetic parameters of anastrozole were described as follows (WinNonLin 5.0.1, Pharsight): The mean value of AUC (ng × h/mL) from time 0 to the last sampling time ($AUC_{0-168\,h}$) was 488.5 for the test and 496.7 for the reference formulation, and AUC from time 0 to infinity ($AUC_{0-\infty}$) was 523.6 for the test and 536.1 for the reference, respectively. The observed maximum plasma concentration (C_{max}, ng/mL) was 10.2 for the test and 9.93 for the reference and the elimination half-life ($T_{1/2}$, h) was 40.3 for the test and 41.3 for the reference, respectively.

4. Conclusions

In the present study, an automated, high-throughput LC–MS/MS method for anastrozole quantitation in human plasma was developed and fully validated. As part of method development, APCI and ESI performance for anastrozole ionization was assessed in terms of sensitivity and specificity. APCI was proven to be 10 times more sensitive than ESI for anastrozole determination as well as 3 times more sensitive for omeprazole determination. APCI was also proven to be more specific and less susceptible than ESI to matrix effects caused by endogenous plasma components. The current study demonstrates the necessity of such a preliminary comparison between the two ion sources at the stage of method development, whenever this is possible and especially when analytes at low concentrations are to be quantified.

APCI LC–MS/MS method sensitivity was further enhanced by the adoption of an ACN protein precipitation step, prior to LLE with MTBE, which resulted in significant peak area increase for both anastrozole and IS. The final analytical protocol involved the lowest plasma volume (190 μL) for analysis, as well as the shortest chromatographic run time (1.6 min) so far reported. The whole sample preparation procedure was highly automated, rapid, and allowed the sensitive, accurate and precise quantitation of anastrozole in human plasma samples from 24 healthy volunteers after the administration of a single dose of 1 mg tablet containing anastrozole, as part of a bioequivalence study, within a 4-day period of time.

References

[1] P.V. Plourde, M. Dyroff, M. Dukes, Breast Cancer Res. Treat. 30 (1994) 103–111.
[2] S.W. Grimm, M.C. Dyroff, Drug Metab. Dispos. 25 (1997) 598–602.
[3] P.E. Lonning, Breast Cancer Res. Treat. 49 (1998) S45–S52.
[4] N. Mauras, K.O. O'Brien, V. Hayes, J. Clin. Endocr. Metab. 85 (2000) 2370–2377.
[5] N. Mauras, J. Lima, D. Patel, A. Rini, E. di Salle, A. Kwok, B. Lippe, J. Clin. Endocr. Metab. 88 (2003) 5951–5956.
[6] G.D. Mendes, D. Hamamoto, J. Ilha, A.S. Pereira, G. De Nucci, J. Chromatogr. B 850 (2007) 553–559.
[7] C. Apostolou, C. Kousoulos, Y. Dotsikas, G.-S. Soumelas, F. Kolocouri, A. Ziaka, Y.L. Loukas, J. Pharm. Biomed. Anal. 46 (2008) 771–779.
[8] K. Liao, B.W. Pack, N.P. Toltl, J. Pharm. Biomed. Anal. 44 (2007) 118–126.
[9] B. Barrett, J. Huclova, V. Borek-Dohalsky, B. Nemec, I. Jelinek, J. Pharm. Biomed. Anal. 41 (2006) 517–526.
[10] E.C. Horning, M.G. Horning, D.I. Carroll, I. Dzidic, R.N. Stillwell, Anal. Chem. 45 (1973) 936–943.
[11] P. Kebarle, M. Peschke, Anal. Chim. Acta 406 (2000) 11–35.
[12] W.M.A. Niessen, J. Cazes (Eds.), Liquid Chromatography–Mass Spectrometry, Marcel Dekker, Incorporation, New York, 1999.

[13] N.B. Cech, C.G. Enke, Mass Spectrom. Rev. 20 (2002) 362–387.
[14] R.E. Ardrey, Liquid Chromatography–Mass Spectrometry: An Introduction, John Wiley & Sons, 2003.
[15] T.L. Constantopoulos, G.S. Jackson, C.G. Enke, J. Am. Soc. Mass Spectrom. 10 (1999) 625–634.
[16] L. Tang, P. Kebarle, Anal. Chem. 63 (1991) 2709–2715.
[17] C.G. Enke, Anal. Chem. 69 (1997) 4885–4893.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882–889.
[19] Y. Hsieh, G. Wang, Y. Wang, S. Chackalamannil, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 16 (2002) 944–950.
[20] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, J. Am. Soc. Mass Spectrom. 14 (2003) 1290–1294.
[21] H. Mei, Y. Hsieh, C. Nardo, X. Xu, S. Wang, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 17 (2003) 97–103.
[22] C. Apostolou, Y. Dotsikas, K. Kousoulos, Y.L. Loukas, J. Chromatogr. B 848 (2007) 239–244.
[23] T.M. Annesley, Clin. Chem. 49 (2003) 1041–1044.
[24] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Vetinary Medicine, 2001. Guidance For Industry. Bioanalytical method validation. http://www.fda.gov/cder/guidance/4252fnl.pdf.