Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 553–559
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Anastrozole quantification in human plasma by high-performance liquid chromatography coupled to photospray tandem mass spectrometry applied to pharmacokinetic studies

Gustavo D. Mendes [a,b], Daniele Hamamoto [a], Jaime Ilha [a,c], Alberto dos Santos Pereira [a], Gilberto De Nucci [a,b,c,*]

[a] *Galeno Research Unit, Latino Coelho St., 1301 Parque Taquaral, 13087-010 Campinas, SP, Brazil*
[b] *Department of Internal Medicine, Faculty of Medical Sciences, State University of Campinas (UNICAMP), P.O. Box 6111, 13083-970 Campinas, SP, Brazil*
[c] *Department of Pharmacology, State University of Campinas, P.O. Box 6111 Campinas, SP, Brazil*

Received 17 December 2005; accepted 24 November 2006
Available online 19 January 2007

Abstract

A rapid, sensitive and specific method for quantifying the aromatase inhibitor (anastrozole) in human plasma using dexchlorpheniramine as the internal standard (I.S.) is described herein. The analyte and the I.S. were extracted from 200 μl of human plasma by liquid–liquid extraction using a mixture of diethyl ether:dichloromethane (70:30, v/v) solution. Extracts were removed and dried in the organic phase then reconstituted with 200 μl of acetonitrile:water (50:50; v/v). The extracts were analyzed by high performance liquid chromatography coupled with photospray tandem mass spectrometry (HPLC–MS–MS). Chromatography was performed isocratically on a Genesis, C18 4 μm analytical column (100 mm × 2.1 mm i.d.). The method had a chromatographic run time of 2.5 min and a linear calibration curve ranging from 0.05–10 ng ml^{-1}. The limit of quantification (LOQ) was 0.05 ng ml^{-1}. This HPLC–MS–MS procedure was used to assess pharmacokinetic studies.

Keywords: Anastrazole; Photospray; Healthy volunteer; Plasma; Pharmacokinetics

1. Introduction

Aromatase inhibitors are a class of compounds which systemically inhibit estrogen synthesis in tissues by inhibiting aromatase, an enzyme that catalyzes the conversion of androgens to estrogen. The link between estrogen and the development and growth of certain breast cancers has long been recognized [1], and substantial evidence indicates that circulating estrogens promote the proliferation of breast cancer. Many current therapies for breast cancer involve hormonal manipulation, with tumour estrogen-deprivation being an established method of treatment [1]. Breast carcinoma has become a major health problem over the past 50 years, affecting as many as one in eight women [2]. Although there have been substantial developments in its treatment, approximately 25% of women with breast carcinoma will eventually die from the disease [3].

Anastrozole, [2,2′-[5-1H-1,2,4-triazole-1-y-methyl)-1,3-phenylene]bis(2-methylpropiononitrile)], is a potent, selective non-steroidal aromatase inhibitor used to treat breast cancer in post-menopausal women [4].

Due to the low dose (1 mg) of anastrozole, the plasma concentration of anastrozole is rather low, which inhibits detection of plasma anastrozole. There are few reports about the determination of anastrozole in biological samples. High-performance liquid chromatography with ultraviolet, fluoresencence or electrochemical detection is not sensitive enough to detect anastrozole in plasma from samples obtained in clinical studies in which standard oral doses have been used. Consequently, anastrazole has been determined in plasma only by capillary gas chromatographic assay with Ni^{63} electron capture detection [5–7].

Here we present a fast, sensitive and selective method for measuring plasma anastrozole by liquid chromatography cou-

* Corresponding author at: 415 Jesuino Marcondes Machado Ave, 13092-320 Campinas, SP, Brazil. Tel.: +55 19 3251 6928; fax: +55 19 3252 1516.
E-mail address: denucci@dglnet.com.br (G. De Nucci).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.044

pled with tandem mass spectrometry (LC–MS–MS), using photospray ionization to quantify anastrozole in human plasma, using dexchlorpheniramine as the internal standard (I.S.). This HPLC–MS–MS procedure was used to assess the pharmacokinetic parameters of the test and reference formulations under comparison in a BA/BE trial.

2. Experimental

2.1. Chemicals and reagents

Anastrozole (99.8%) was provided by Astrazeneca (lot number 012APM, Cheshire, England), and dexchlorpheniramine (99.9%) was obtained from Kongo Chemical Co. (lot number 25040, Toyama, Japan), Acetonitrile and methanol (HPLC-grade), diethyl ether and hexane (analysis grade) were purchased from Mallinckrodt (Paris, ST, USA). Ultra-pure water was obtained from an Elga UHQ system (Elga, UK). Blank human blood was collected from healthy drug-free volunteers by Hemocentro of Campinas, Brazil. Plasma was obtained by centrifugation of blood treated with anticoagulant sodium heparin. Pooled plasma was prepared and then stored at approximately −20 °C until needed.

2.2. Calibration standards and quality control

Stock solutions of anastrozole and internal standard (dexchlorpheniramine) were prepared in acetonitrile:water (50:50 v/v) at concentrations of 1 mg ml^{-1}. Calibration curves of anastrozole were prepared by spiking blank plasma at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 ng ml^{-1}. The analysis was carried out in duplicate for each concentration. The quality control samples were prepared in blank plasma at concentrations of 0.15, 0.6 and 9.0 ng ml^{-1} (QCL, QCM and QCH, respectively). The spiked plasma samples (standards and quality controls) were extracted from each analytical batch along with the unknown samples. For analytical runs requiring sample dilutions, a fourth quality control was included, prepared in blank plasma at a concentration of 18.0 ng ml^{-1} and then diluted 1:1 (v/v).

2.3. Sample preparation

All frozen human plasma samples were previously thawed at room temperature and centrifuged at 2000 × *g* for 1 min at 4 °C to precipitate solids. Two-hundred microliters of sample human plasma was introduced into glass tubes followed by addition of 50 μl of the internal standard solution (10 ng ml^{-1} of Dexchlorpheniramine in acetonitrile:water 50/50; v/v solution); then, samples were vortex-mixed for approximately 15 s. Diethyl ether/dichloromethane (70/30; v/v) was added (4 ml) to all tubes, and the extraction was performed by vortex-mixing for 40 s. The upper organic phase was transferred to another set of clean glass tubes and evaporated to dryness under N_2 at 40 °C. The dry residues were dissolved with 200 μl of a solution of acetonitrile:water (50:50, v/v). Vials were capped and then placed into the autosampler.

When sample dilutions were applied, one-hundred microliters of sample human plasma was introduced into glass tubes followed by addition of one-hundred microliters of blank human plasma and of 50 μl of the internal standard solution.

2.4. Chromatographic conditions

A 10 μl aliquot of each plasma extract was injected into a Genesis C18 4 μm analytical column (100 mm × 2.1 mm i.d.) operating at room temperature. The compounds were eluted by pumping the mobile phase acetonitrile:methanol:water:acetone (60:20:15:5, v/v/v/v) containing 0.1% of acetic acid and 10 mM of ammonium acetate at a flow-rate of 0.450 ml min^{-1} using a Shimadzu HPLC pump (model LC10AD, Shimadzu, Japan). The mobile phase was prepared by adding 1 ml acetic acid and 0.77 g of ammonium acetate into a measuring cylinder (500 ml capacity) containing 150 ml of water. Separately, 200 ml of methanol was added to a measuring cylinder of 500 ml; 600 ml of acetonitrile was added into a measuring cylinder of 1000 ml, and, finally, 50 ml of acetone was added into a measuring cylinder of 100 ml. These solutions were carefully mixed into a 1000 mL Erlenmeyer, allowing vapours to vent between shaking.

Under these conditions, typical standard retention times were 0.9 ± 0.1 (mean ± SD) min for anastrozole and 1.3 ± 0.2 (mean ± SD) min for dexchlorpheniramine, and back-pressure values of approximately 60 bar were observed. Temperature of the auto-sampler was kept at 20 °C and run-time was set to 2.5 min.

2.5. Mass-spectrometric conditions

Mass spectrometry was performed in a Sciex API 3000 triple stage quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a photospray source operating in positive mode. The API 3000 was set up for Multiple Reaction Monitoring (MRM) to monitor the transitions 294.0 > 225.2 and 275.0 > 230.1, for anastrazole and the internal standard, respectively. The source block temperature was set at 400 °C and the photoionization capillary voltage to 1.5 kV. Nitrogen was used as collision gas and acetone was used as dopant into the mobile phase. For both anastrozole and internal standard, the following optimized parameters were obtained: for anastrozole the declustering potential, focusing potential, collision energy and collision exit potential were 41 (V), 110 (V), 35 (eV) and 16 (V), respectively. The corresponding values for internal standard were 26 (V), 170 (V), 29 (eV) and 22 (V), respectively. Data were acquired by Analyst software (1.3.4, Applied Biosystems, Foster City, CA, USA).

2.6. Sample collected

Blood samples (4 ml) from a suitable antecubital vein were collected by an indwelling catheter into heparin containing tubes prior to administration and at 20 min, 40 min, 1 h, 1 h 20 min, 1 h 40 min, 2 h, 2 h 20 min, 2 h 40 min, 3 h, 3 h 30 min, 4 h, 4 h 30 min, 5 h, 5 h 30 min, 6 h, 8 h, 10 h, 12 h, 18 h, 24 h, 48 h, 96 h and 120 h after administration the of 1 mg anastrozole tablet

formulation. Blood samples were centrifuged at approximately $2000 \times g$ for 10 min at room temperature and the plasma was stored at −20 °C until assayed for anastrozole content.

2.7. Linearity

Linearity was determined to assess the performance of the method. A linear least-squares regression with a weighting index of $1/x^2$ order was performed on the peak-area ratios of anastrozole and I.S. versus Anastrozole concentrations of the eight plasma standards (0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 ng ml^{-1}) in duplicate to generate a calibration curve. Unknown sample peak-area ratios were then interpolated from the calibration curve to provide concentrations of anastrozole.

2.8. Specificity and selectivity

Blank samples from five different pools of plasma, including one lipemic and one haemolyzed, were tested for interference using the proposed extraction procedure and analytical conditions.

2.9. Recovery

The recovery was evaluated by calculating the mean (and RSD) of the response of each concentration and dividing the extracted sample mean response by the unextracted (spiked blank plasma extract) sample mean of the corresponding concentration. Comparison with the unextracted samples, spiked on plasma residues, was performed in order to eliminate the matrix effect, providing a true recovery. Matrix effect experiments were carried out using the ratio between spiked mobile phase solutions and unextracted samples, spiked on plasma residues.

2.10. Stability

Stability quality control plasma samples (0.15, 0.6 and 9.0 ng ml^{-1}) were subjected to short-term (6 h) room temperature, three freeze/thaw (−15 to 25 °C) cycles, 28 h autosampler stability (8 °C) and long-term stability at −15 °C (25 days) tests. Subsequently, the anastrozole concentrations were measured compared to freshly prepared samples.

2.11. Precision and accuracy

To assess precision and accuracy of the developed analytical method, four distinct concentrations in the range of expected concentrations were evaluated using eight determinations per concentration.

Precision and accuracy was assessed at within-day basis (intra-batch) during a single analytical run and at a between-day basis (inter-batch), which measures the between day variability, possibly involving different analysts and regents.

2.12. Dilution

To evaluate the applicability of the developed analytical method for the assay of diluted samples (1:1 dilution), in order to extend the quantification range to 20 ng ml^{-1}, a precision and accuracy test was performed using specific quality control samples spiked with Anastrozole, 18.0 ng ml^{-1} and then diluted 1:1 (v/v) with blank plasma.

Precision and accuracy was assessed at within-day basis (intra-batch) and at a between-day basis (inter-batch), following the same procedures and conditions applicable for the evaluation of normal range quality controls. Three distinct analytical runs were evaluated, using eight determinations, each.

2.13. Ionic suppression

A procedure to assess the effect of ion suppression on the MS/MS was performed. The experimental set-up consisted of an infusion pump connected to the system by a "zero volume tee" before the split and the HPLC system pumping the mobile phase, which was the same as that used in the routine analysis of anastrozole, i.e. acetonitrile:methanol:water:acetone (60:20:15:5, v/v/v/v) containing 0.1% of acetic acid and 10 mM of ammonium acetate at a flow-rate of 0.450 ml min^{-1}. The infusion pump (Harvard Apparatus, Holliston, MA, USA) was set to transfer (10 μl min^{-1}) a mixture of analyte and internal standard in mobile phase (both 1 μg ml^{-1}). A sample of human pooled blank plasma was extracted and the reconstituted extract was injected into the HPLC system while the standard mixture was being infused. In this system any ion suppression would be observed as a depression of the MS signal.

2.14. Pharmacokinetics and statistical analysis

The first-order terminal elimination rate constant (ke) was estimated by linear regression from the points describing the elimination phase in a log-linear plot. Half-life ($t_{1/2}$) was derived from this rate constant ($t_{1/2} = \ln(2)\,\mathrm{ke}^{-1}$).

The areas under the Anastrozole plasma concentration versus. time curves from 0 to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity (AUC_{0-inf}) was done by adding the value C_{last}/ke to the calculated AUC_{last} (where C_{last} = the last detectable concentration).

Software used included WinNonLin Professional Network Edition (v. 1.5) in single compartmental model for extra vascular administration, Microsoft Excel (v. 7.0) and GraphPad Prism (v. 3.02).

3. Results and discussion

3.1. Method development

In atmospheric pressure photoionization, toluene is a dopant typically used due to its ionization potential of 8.83 eV, as well as its potential high purity grade and low toxicity. Acetone (ionization potential of 9.70 eV) is another substance which can be used as dopant. However, toluene and acetone were compared in the analysis of free anabolic steroids and, with toluene, approximately 20–50% higher sensitivity was achieved [8]. In

our laboratory, acetone has been used routinely as dopant in several methods using positive or negative ionization mode in the analysis for different substances, as for example, isosorbide 5-mononitrate [9]. Although the literature indicates better results using toluene as compared to acetone, the use of acetone (5% of the mobile phase volume) as dopant has practical advantages since it can be used in the mobile phase without HPLC column damage, while maintaining a high throughput (more than 200 samples/day can be analyzed) and a rugged high sensitivity. In this study, the use of APPI with acetone as dopant permitted a limit of quantification (LOQ) of 0.05 ng ml^{-1} of anastrozole in plasma). The mass chromatograms of a blank and LOQ samples are shown in Fig. 1 in which the retention times for anastrozole and I.S. were 0.91 and 1.30 min, respectively. During the pre-validation studies, the APPI and ESI sources were used in the same mass spectrometry and the results showed that both source systems exhibited good specificity without matrix interferences. However, the LC-APPI-MS/MS is a more sensitive system for anastrazole analysis. The APPI increased the sensitivity by 2-fold when compared with ESI (Fig. 1). This result is consistent with previous literature data suggesting that APPI is a highly promising technique in high-throughput pharmaceutical analysis and provides superior performance in ionization neutral compounds over electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), while providing comparable ionization for polar compounds [12].

Due to the high intensities of the *m/z* 294.0 → 225.2 (anastrozole) and *m/z* 275.0 → 230.1 (I.S.) transitions (Fig. 2) and no detectable interference in blank human plasma samples, these transitions were used in the present study. The proposed fragmentation pathways for anastrozole and for dexchlorpheniramine are presented in Figs. 3 and 4.

3.2. Assay performance

The optimized method was validated by the assessment of recovery, linearity, limit of quantification, precision and accuracy (Table 1). A deviation from the actual value and a relative standard deviation (RSD) within 15% were allowed, except for the LOQ, whose values were allowed to deviate by no more than 20%, as recommended by Shah et al. [10] and Bressole et al. [11] for the analysis of biological samples for pharmacokinetic studies.

The simplest regression method for the calibration curves of the anastrozole was $y = a + bx$ from 0.05 to 10 ng ml^{-1} (calibration curve $y = mx + b$; $m = 0.0673 \pm 0.0042$ and $b = 0.000485 \pm 0.000078$, $r^2 > 0.9989$). A linear least-squares regression with weighting index of $1/x^2$ was carried out on the peak area ratios of anastrozole and I.S. versus anastrozole concentrations of the eight human plasma standards (in duplicate) to generate a calibration curve. In the case of anastrozole and its internal standard, dexchlorpheniramine, there was no significant ion suppression in the region where the analyte and internal standard were eluted.

The recovery of anastrozole was 59.2% (RSD 7.9%), 72.6% (RSD 4.4%) and 73.0% (RSD 2.4%) for the 0.15, 0.6 and 9.0 ng ml^{-1} standard concentrations, respectively. The recov-

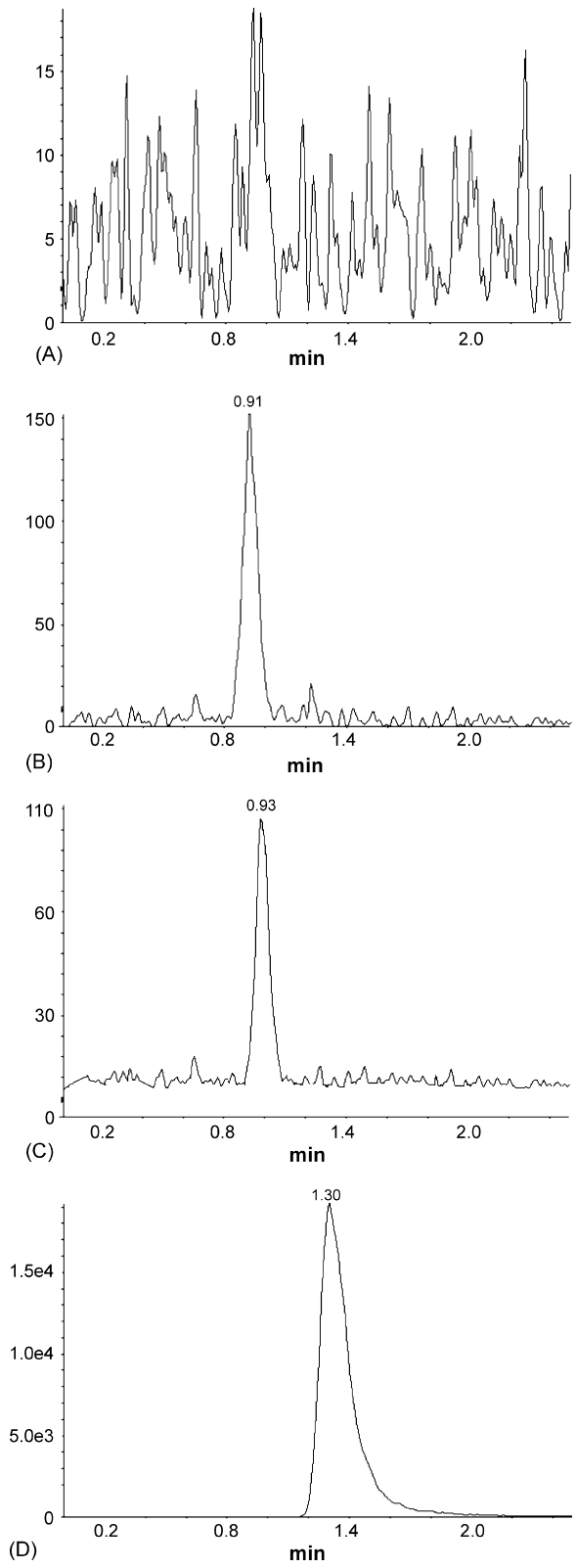

Fig. 1. (A) MRM chromatogram (294.0 → 225.2) of blank pooled human plasma for the anastrazole. (B) MRM chromatogram (294.0 → 225.2) of the anastrazole spiked in human plasma at a final concentration of 50 pg ml^{-1}. (C) MRM chromatogram (294.0 → 225.2) of the anastrazole spiked in human plasma at a final concentration of 50 pg ml^{-1} using the source turboionsray. (D) MRM chromatogram (75.0 → 230.1) of the dexchlorpheniramine spiked in human plasma at a final concentration of 2.5 ng ml^{-1}.

Table 1
Quality controls (QC) validating the accuracy and precision of the anastrazole

	Parameter	Nominal concentration (ng ml^{-1})				
		0.050	0.15	0.60	9.0	18.0[a]
Intra-batch	Mean found ($n=8$) (ng ml^{-1})	0.0536	0.171	0.678	10.3	19.8
	Precision (%)	13.3	4.4	6.1	5.1	6.0
	Accuracy (%)	107.1	114.2	113.0	114.9	110.0
Inter-batch	Mean found ($n=3$) (ng ml^{-1})	0.0541	0.172	0.670	10.3	19.6
	Precision (%)	15.4	5.8	5.8	4.3	5.0
	Accuracy (%)	108.3	114.5	111.7	114.3	108.9

[a] This quality control sample was used for the assay of diluted samples.

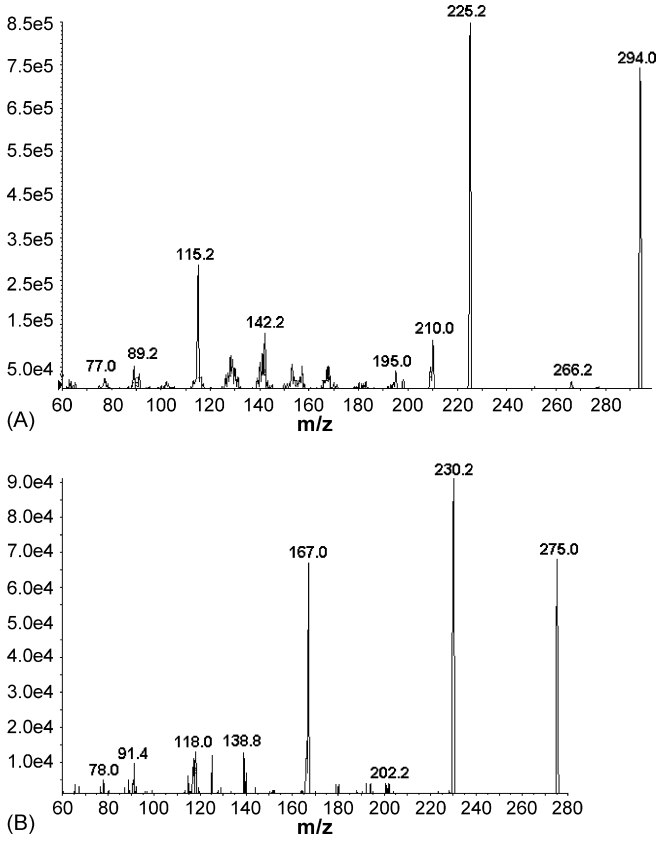

Fig. 2. The product ion mass spectra of (A) Anastrozole and (B) Dexchlorpheniramine.

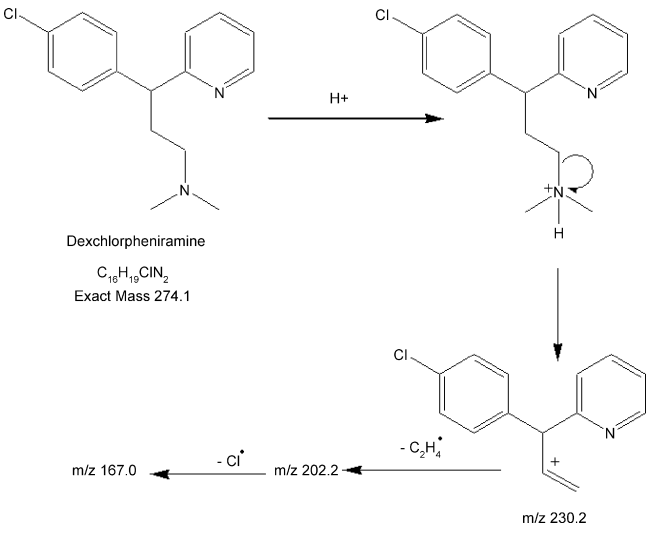

Fig. 4. Proposed fragmentation pathways for the Dexchlorpheniramine (B).

ery of I.S. was 81.5% (RSD 14.6%), 76.3% (RSD 3.0%) and 75.2 (RSD 1.1%) for the 0.15, 0.6 and 9.0 ng ml^{-1} standard concentrations, respectively. The liquid–liquid extraction employed was chosen because it is faster, cheaper and has an appropriate recovery with a low variability. During the pre-validation assays, different solutions were tested for liquid–liquid extraction and the best result, when considering a better recovery and lower interference, was of diethyl ether: dichloromethane (70:30, v/v), which also presented no significant matrix effect.

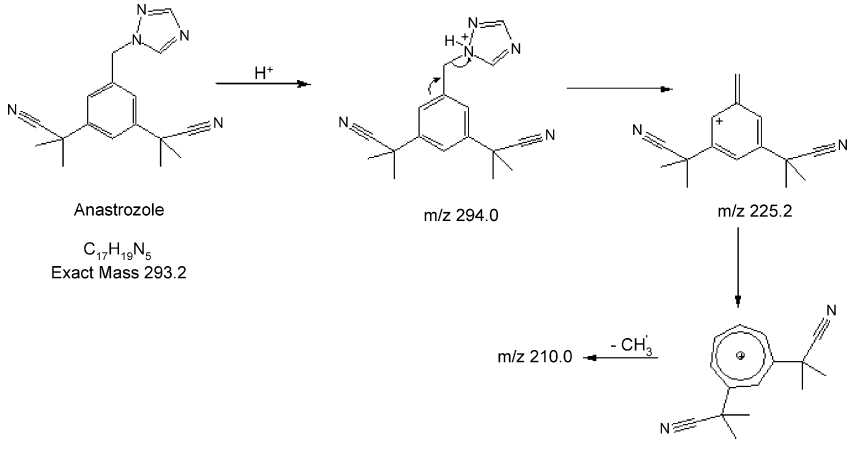

Fig. 3. Proposed fragmentation pathways for the Anastrozole (A).

Table 2
Stability test for anastroloze

	Low sample		Medium sample		High sample	
	Reference values	Values after 28 h	Reference values	Values after 28 h	Reference values	Values after 28 h
Post-processing stability test						
Mean	0.15	0.15	0.57	0.55	8.40	8.20
CV (%)	4.10	5.10	3.00	4.30	2.60	3.30
Variation	1.90		−4.70		−2.30	
	Low sample		Medium sample		High sample	
	Reference values	Values after 3 cycles	Reference values	Values after 3 cycles	Reference values	Values after 3 cycles
Freeze-and-thaw stability test						
Mean	0.16	0.13	0.57	0.53	8.87	0.16
CV (%)	8.90	6.90	9.80	2.00	6.60	8.90
Variation	−16.90		−6.50		−1.70	
	Low sample		Medium sample		High sample	
	Reference values	Values after 6 h	Reference values	Values after 6 h	Reference values	Values after 6 h
Short-term stability test						
Mean	0.16	0.14	0.57	0.55	8.87	7.94
CV (%)	8.90	10.20	9.80	4.00	6.60	6.60
Variation	−12.20		−3.10		−10.50	
	Low sample		Medium sample		High sample	
	Reference values	Values after 25 days	Reference values	Values after 25 days	Reference values	Values after 25 days
Long term stability test						
Mean	0.16	0.16	0.63	0.66	8.79	8.96
CV (%)	4.20	3.90	6.40	5.50	1.70	5.40
Variation	−0.20		4.20		1.90	

The stability tests (Table 2) performed, indicated no significant degradation under the conditions described above, including the freeze and thaw, short-term at room temperature and the long term (25 days) tests. Human plasma spiked at final concentrations of 0.15, 0.60 and 9.0 ng ml^{-1} ($n = 5$ for each concentration) were tested.

The HPLC-MS/MS method described herein for anastrozole quantification in human plasma is applicable to analytical runs lasting 24 h (or less) as observed in the post-processing tests and offers advantages over methods previously reported: the present method is ten times more sensitive, while the analytical run is four times faster, permitting a high throughput.

Table 3 shows the values for pharmacokinetic parameters after a single oral dose of anastrozole formulation (Fig. 5). Anastrozole peak plasma concentration (C_{max}) values and the time values achieved (T_{max}) were similar to those reported in the literature by Yuan et al. [6]. The high sensitivity of the method allows for a better evaluation of pharmacokinetic parameters, mainly of K_e, $T_{1/2}$ and AUC_{inf}. The $AUC_{extrapolated}$ demonstrates that samples collected until $AUC_{(0–Tlast)}$ reach 80% of $AUC_{(0–infinite)}$ [13].

Table 3
Pharmacokinetic parameters obtained from one volunteer after administration of 1 mg anastrozole tablet formulation

Pharmacokinetics parameters for anastrazole	
$AUC_{0–inf}$ ([ng h]/ml^{-1})	629.20
$AUC_{0–Tlast}$ ([ng h]/ml^{-1})	536.61
$AUC_{extrapolated}$ (%)	14.71
C_{max} (ng ml^{-1})	11.40
T_{max} (h)	0.67
$T_{1/2}$ (h)	42.50
T_{last} (h)	120.00
K_e (h^{-1})	0.016

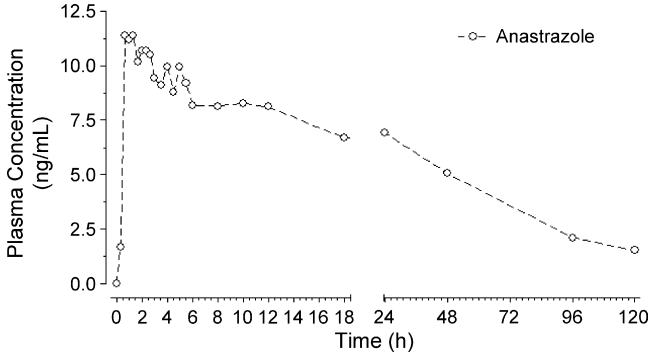

Fig. 5. Anastrozole plasma mean concentrations vs. time profile obtained after the single oral administration of 1 mg of Anastrozole formulation.

4. Conclusion

This paper reports for the first time on a novel method to measure anastrozole by HPLC–MS–MS and for quantification

of this aromatase inhibitor in human plasma. This method offers advantages over those previously reported, in terms of both a simple liquid–liquid extraction without clean-up procedures, as well as a faster run time (2.5 min). The LOQ of 50 pg ml^{-1} is well suited for pharmacokinetic studies. The assay performance results indicate that the method is precise and accurate enough for the routine determination of the anastrozole in human plasma.

Acknowledgements

This pharmacokinetic trial was funded by AstraZeneca.

References

[1] M. Baum, Br. J. Cancer 85 (Suppl 2) (2001) 15.
[2] A.U. Buzdar, J.F.R. Robertson, W. Eiermann, J.M. Nabholtz, Am. Cancer Soc. 95 (2002) 2006.
[3] H.L. Howe, P.A. Wingo, M.J. Thum, J. Natl. Cancer Inst. 93 (2001) 824.
[4] P.V. Plourde, M. Dyroff, M. Dukes, Breast Cancer Res. Treat. 30 (1994) 103.
[5] M.J. Bock, I. Bara, N. LeDonne, A. Martz, M. Dyroff, J. Chromatogr. B 700 (1997) 131.
[6] J. Yuan, P.Q. Wang, S.R. Ge, F.R. An, A.G. Shi, J. Chen, J.Y. Liang, Acta Pharmacol. Sin. 22 (2001) 573.
[7] G. Duan, J. Liang, M. Zuo, Biomed. Chromatogr. 16 (2002) 400.
[8] A. Leinonen, T. Kuuranne, R. Kostiainen, J. Mass Spectrom. 37 (2002) 693.
[9] L.C. Silva, L.S.O.B. Oliveira, G.D. Mendes, G. Garcia, A.S. Pereira, De Nucci, J. Chromatogr. B 832 (2006) 302.
[10] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGlivery, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pitman, S. Spector, Pharm. Res. 9 (1992) 588.
[11] F. Bressole, M.B. Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[12] Y. Cai, D. Kingery, O. McConnell, A.C. Bach II, Rapid Commun. Mass Spectrom. 19 (2005) 1717.
[13] FDA/CDER, Guidance for Industry – Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations, Revision 1, March 2003.