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ORIGINAL RESEARCH

A liquid chromatography–mass spectrometry method for the quantification of both etoricoxib and valdecoxib in human plasma

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ABSTRACT: A practicable and selective liquid chromatography–mass spectrometry assay for the determination of two cyclooxygenase-2 inhibitors, etoricoxib and valdecoxib, in human plasma is presented. The analytical technique is based on reversed-phase high-performance liquid chromatography (HPLC) coupled to atmospheric pressure chemical ionisation (APCI) mass spectrometry (Finnigan Mat LCQ ion trap). Mass analysis was performed in the positive ion mode. The ion trap was operated in the tandem MS mode (MS^2) and the transitions of etoricoxib (m/z 359.2 → 280.3) and valdecoxib (m/z 315.1 → 235.1) were followed by selected reaction monitoring. Retention times of etoricoxib and valdecoxib were 1.05 and 1.08 min, respectively. The method was validated over a linear range 10–2500 and 5–1000 µg/L using the other substrate as internal standard. After validation, the method was used to study the pharmacokinetic profile of etoricoxib or valdecoxib in a healthy volunteer after administration of a single oral dose (valdecoxib, 20 mg; etoricoxib, 90 mg). The presented method was sufficient to cover more than 90% of the area under the plasma concentration time curve. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: cyclooxygenase-2 inhibitors; HPLC/APCI MS; pharmacokinetic profiles

INTRODUCTION

Etoricoxib [5-chloro-3-(4-methanesulphonylphenyl)-6′methyl-[2,3′]-bipyridinyl, ARCOXIA®, MSD] and valdecoxib [4-(5-methyl-3-phenylisoxazol-4-yl) benzenesulfonamide, BEXTRA®, Pfizer Inc.; Fig. 1] are new non-steroidal anti-inflammatory drugs which are selective inhibitors of the cyclooxygenase-2 (COX-2) enzyme. Both compounds have recently been approved for treatment of osteoarthritis. Moreover, valdecoxib has been launched for rheumatoid arthritis and dysmenorrhea. The enzyme cyclooxygenase is present in two isoforms, referred to as COX-1 and COX-2. Whereas COX-1 is constitutively expressed in nearly all tissues, COX-2 is induced at sites of pain and inflammation (Needleman and Isakson, 1997; Golden and Abramson, 1999). Therefore, selective inhibition of COX-2 should maintain the anti-inflammatory effects while reducing adverse effects (gastrointestinal adverse effects, bleeding) as compared with traditional non-steroidal agents that inhibit both COX-1 and COX-2

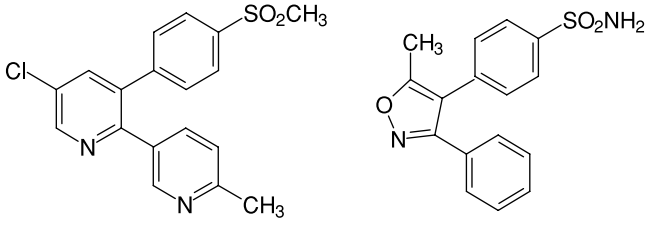

Figure 1. Chemical structures of etoricoxib and valdecoxib.

(Fitzgerald and Patrono, 2001). Etoricoxib and valdecoxib are COX-2 inhibitors which are more selective than the formerly approved substances celecoxib or rofecoxib (Riendeau *et al.*, 2001; Capone *et al.*, 2003; Brune and Hinz, 2004).

Since COX-2 inhibitors are increasingly prescribed drugs, there is a growing interest in their quantification. In the literature, several high-performance liquid chromatography (HPLC) assays with different detection methods have been published. An analytical method for etoricoxib using post-column photochemical cyclization and fluorescence detection was described (Matthews *et al.*, 2001). However, this assay represents a time-consuming approach. Moreover, two different LC-MS-MS methods have been recently published, involving electrospray ionization (Brautigam *et al.*, 2003) or using a heated nebulizer interface (Rose *et al.*, 2002). For quantification of valdecoxib in plasma only one LC-MS-MS method with negative electrospray

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Abbreviations used: COX-2, cyclooxygenase-2; LOQ, limit of quantification; MSD, 5-chloro-3-(4-methanesulphonylphenyl)-6′methyl-[2,3′]-bipyridinyl.

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ionization has been published (Zhang *et al.*, 2003). We developed a rapid and selective LC-MS-MS method for both COX-2 inhibitors etoricoxib and valdecoxib. We used APCI detection after a very easy liquid–liquid extraction. In contrast to the other methods, we used a Finnigan Mat LCQ ion trap spectrometer. Finally, a method has been established for plasma specimens sensitive enough to monitor etoricoxib and valdecoxib after oral administration of the normal daily recommended dose.

EXPERIMENTAL

Reagents and solutions. Etoricoxib and valdecoxib were kindly supplied by S. Laufer (Institute of Pharmacy, Eberhard-Karls-University of Tübingen, Germany). All other reagents were purchased from Merck (Darmstadt/Germany) in analytical grade. Methanol and acetonitrile were of HPLC grade.

The stock solutions of etoricoxib and valdecoxib (50 mg/L) were prepared with acetonitrile:water (50:50, v/v) and were stored at −80°C for no longer than 2 months.

Figure 1 shows the structural formula of the used COX-2 inhibitors. All standard solutions were found to be stable at −80°C for at least 2 months and for a minimum of 2 weeks when stored protected from light at ambient temperature.

Sample preparation. An aliquot of plasma sample (1.00 mL), 100 µL acetonitrile:water (50:50, v/v), and 100 µL internal standard solution (valdecoxib, 4 µg/mL, etoricoxib, 5 µg/mL) were vortexed for approximately 1 min. Afterwards, 1 mL 0.1 M sodium acetate buffer (pH 5) and 4 mL dichloromethane:hexane (50:50, v/v) were added. The tubes were capped, agitated in an overhead shaker for 15 min (30 min^{-1}) and centrifuged at 4000***g*** for 10 min. The organic layer (3.5 mL) was transferred into another tube. The solvent was evaporated under a stream of nitrogen at 40°C. The resulting residue was reconstituted in 500 µL of mobile phase and aliquots of 20 µL (etoricoxib) or 100 µL (valdecoxib) injected onto the column. Plasma calibration standards were prepared in the same manner, from 100 µL of acetonitrile containing etoricoxib or valdecoxib to achieve different final concentrations (etoricoxib, 10, 25, 50, 75, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250 and 2500 µg/L; valdecoxib, 5, 10, 25, 50, 75, 100, 200, 300, 500, 750 and 1000 µg/L).

Instrumentation. The HPLC system consisted of a PU-1585 pump and an AS-1550 auto-injector (Jasco, Groß-Umstadt/Germany). Masses were acquired on a Finnigan MAT LCQ ion trap spectrometer equipped with an APCI interface (Thermoquest, Egelsbach/Germany) and connected to a PC running the standard software Navigator (1.2.).

Liquid chromatography–mass spectrometry. HPLC was carried out isocratically at ambient temperature using a Nucleosil C8 guard column (120–5, 8 × 3 mm; Macherey & Nagel, Düren, Germany), and an eluent comprising methanol: water (50:50, v/v) and 1% acetic acid at a flow rate of 300 µL/min, with the outlet coupled to the mass spectrometer's APCI (atmospheric pressure chemical ionisation) source.

The vaporizer temperature was set to 450°C and N_2 was applied as the sheath and auxiliary gas at flow rates (arbitrary units) of 30 (etoricoxib) or 60 and 0 (valdecoxib), respectively.

The heated capillary was maintained at 145°C. Mass analysis was performed at unit resolution in the positive ion mode with the corona discharge current set to 5 µA, and the potentials of tube lens and capillary to 45 and 15 (etoricoxib) or 35 and 8 V (valdecoxib), respectively. The ion trap was operated in the on-line tandem MS mode (MS^2) and the transitions of etoricoxib [parent m/z 359.2, CID (collision-induced dissociation) energy 30%, product m/z 280.3] and valdecoxib (315.1, 24%, 235.1) were followed by selected reaction monitoring (SRM) with an maximum automatic gain control (AGC) ion storage time of 500 ms and a mass isolation width of 2 u; three microscans were collected per spectrum.

The peak area ratios of the analyte and the internal standard were calculated. For the validation of the method as well as quantitative analysis of the substrate the relevant principles (Shah *et al.*, 2000) were taken into account. The standard functions were plotted with the following concentrations: 10, 25, 50, 75, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250 and 2500 µg/L for quantification of etoricoxib and 5, 10, 25, 50, 75, 100, 200, 300, 500, 750 and 1000 µg/L for quantification of valdecoxib. The intra-day repeatability of the method was tested by multiple analysis of individual human plasma samples on the same day. Inter-day reproducibility was assessed on three different days. The recovery of etoricoxib and valdecoxib was determined at 10, 750, 2500 and 5, 100, 1000 µg/L by comparing the peak area after extraction of human plasma standards with the peak area obtained from injection of the same amount of etoricoxib and valdecoxib aqueous standards.

Patients and collection of plasma. A single oral dose of etoricoxib (ARCOXIA® 90 mg tablet) or valdecoxib (BEXTRA® 20 mg tablet) was administered to a healthy male volunteer. EDTA blood samples (5 mL) were taken before and 0.5, 1, 2, 3, 4, 6, 8, 24 and 51 (etoricoxib only) h after administration and centrifuged immediately. The plasma samples were stored at −80°C for no longer than 2 months. Informed consent was obtained from the study participant according to the Declaration of Helsinki.

Pharmacokinetic methods. Plasma concentration–time curves were evaluated by non-compartmental analysis using WinNonline, version 3.3 (Pharsight Corp, Mountain View, CA, USA). The apparent half-life, $t_{1/2,\lambda z}$, was calculated as $\ln 2/\lambda_z$, where λ_z denotes the time constant of the terminal slope. The area under the plasma concentration–time curve after an oral dose (AUC_{or}) was calculated using the linear trapezoidal rule. The extrapolated AUC_∞ after the last observed plasma concentration was obtained by dividing this plasma concentration by λ_z.

RESULTS

The described method yields reliable results for the quantitative analysis of etoricoxib and valdecoxib

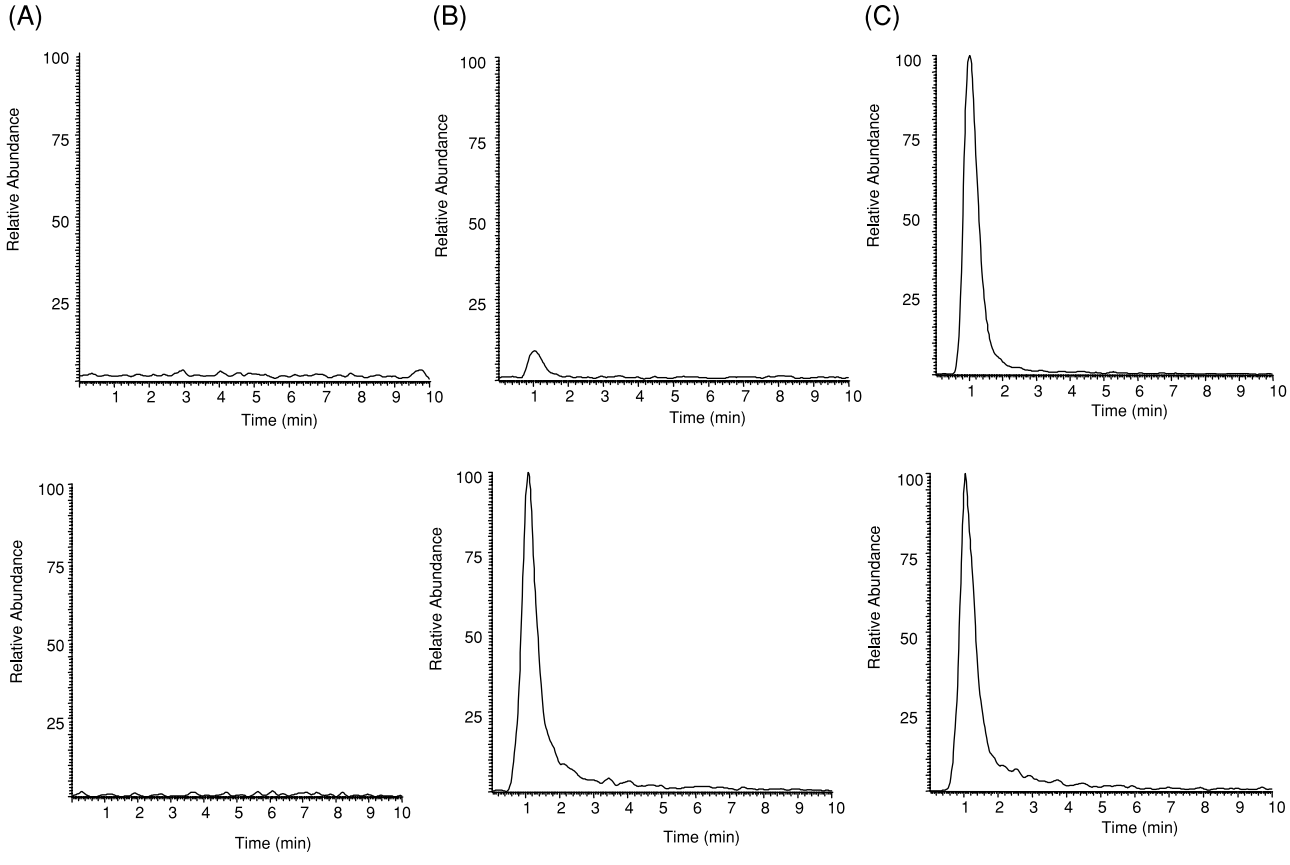

Figure 2. Etoricoxib: representative SRM chromatogram of human plasma. (A) Lacking both etoricoxib and valdecoxib, (B) spiked with 10 μg/L etoricoxib (LOQ) and internal standard valdecoxib (500 μg/L); (C) sample 51 h after oral administration of 90 mg etoricoxib (133.5 μg/L).

within 5 min (Figs 2 and 3). The retention times of etoricoxib and valdecoxib were 1.05 and 1.08 min, respectively. When plasma specimens without the addition of etoricoxib or valdecoxib were analysed, the chromatograms contained no spurious signals in the relevant portions of the trace [Figs 2(A) and 3(A)]. Chromatograms obtained from 20 different human plasma samples showed that the method is adequately specific. Furthermore, plasma samples spiked with common analgesics (diclofenac, flurbiprofen, ibuprofen, indomethacin, acetaminophen, acetylsalicylic acid, ketoprofen and phenylbutazone) were analysed. None of these substances interfered with the method for quantification of etoricoxib or valdecoxib. Three separate analytical series analysed in duplicate were used to verify linearity of the calibration curve for the relevant range 10–2500 μg/L (etoricoxib) or 5–1000 μg/L (valdecoxib) in human plasma. The correlation coefficients (r^2) were greater than 0.995 in each case.

Assay checks continued for 2 months demonstrated that the stability of etoricoxib and valdecoxib in human plasma at three concentrations (etoricoxib, 10, 750, 2500 μg/L; valdecoxib, 5, 100, 1000 μg/L, $n = 5$ in each case), and the standard solution of etoricoxib and valdecoxib (50 mg/L acetonitrile) stored at −80°C, including two freeze–thaw cycles, was adequate (difference between measured value before and after freeze–thaw cycles <10% in each case).

The intra- and inter-day assay precision and accuracy for low, medium and high concentrations of etoricoxib and valdecoxib in human plasma are summarized in Table 1. The recoveries at concentrations 10, 750 and 2500 μg/L of etoricoxib were 75.9 ± 7.2, 84.4 ± 2.1 and 88.5 ± 2.4%, and 5, 100 and 1000 μg/L of valdecoxib were 80.1 ± 3.5, 80.1 ± 8.6 and 82.2 ± 6.1%. The limit of quantification (LOQ), according to Shah *et al.* (2000) (CV, accuracy <20%), was 10 and 5 μg/L, respectively [Figs 2(B) and 3(B), Table 1].

The validated method has been successfully used to study the pharmacokinetic profile of etoricoxib and valdecoxib after a single oral dose. For these measurements, the calibration curves were established daily using various plasma concentrations (etoricoxib, 10, 25, 50, 75, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250 and 2500 μg/L; valdecoxib, 5, 10, 25, 50, 75, 100, 200, 300, 500, 750 and 1000 μg/L). Furthermore, 10% of the measured samples were quality-control (QC) samples. QC samples were incorporated in duplicate in

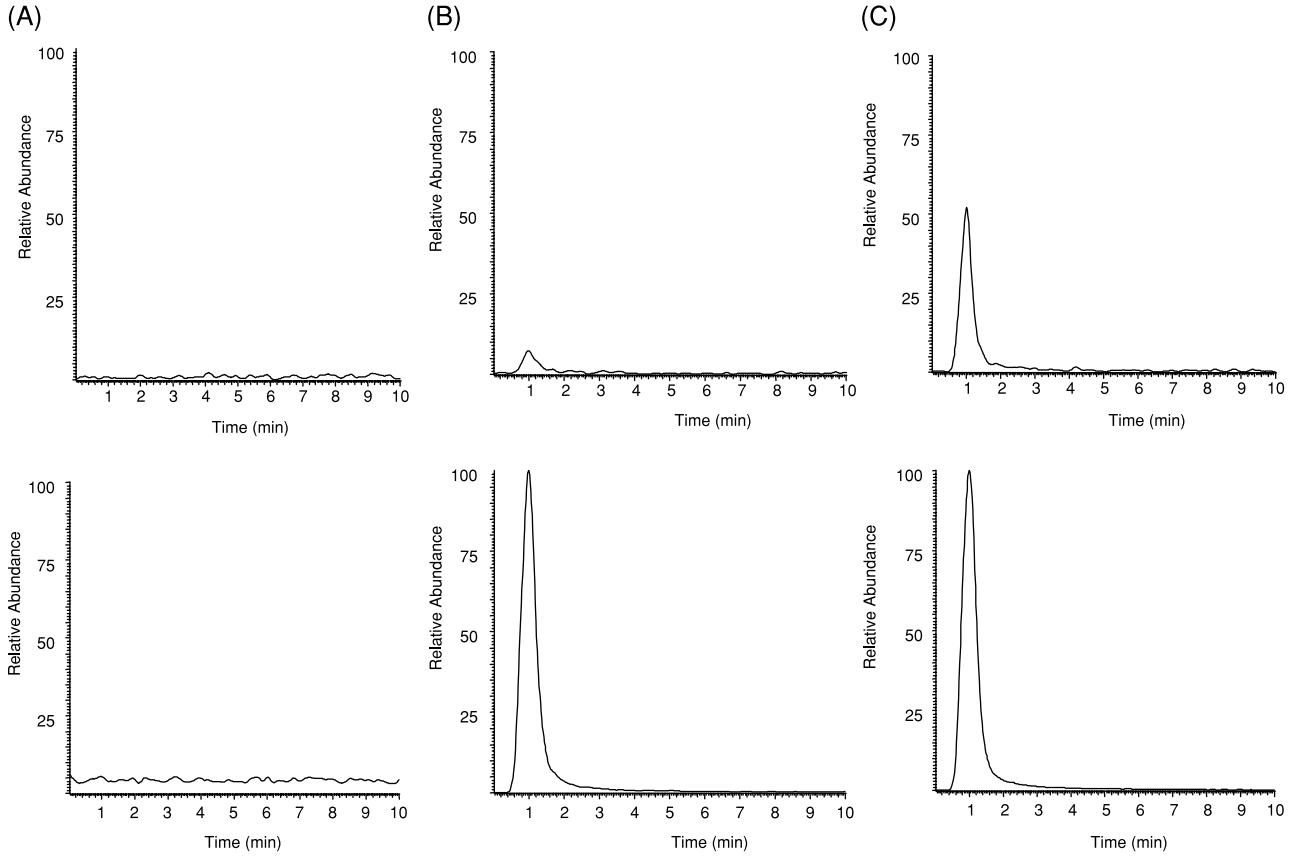

Figure 3. Valdecoxib: representative SRM chromatogram of human plasma. (A) Lacking both valdecoxib and internal standard (etoricoxib); (B) spiked with 5 μg/L valdecoxib (LOQ) and internal standard etoricoxib (400 μg/L); (C) sample 24 h after oral administration of 20 mg valdecoxib (34.5 μg/L).

Table 1. Precision (coefficient of variations, CV) and accuracy for low, medium and high concentrations of etoricoxib and valdecoxib in human plasma

	Intra-day (n = 5)			Inter-day (three days, n = 5 each)		
Added concentration (μg/L)	Measured concentration (mean ± SD, μg/L)	CV (%)	Accuracy (%)	Measured concentration (mean ± SD, μg/L)	CV (%)	Accuracy (%)
Etoricoxib						
10	9.5 ± 0.3	3.1	−4.5	9.5 ± 0.5	5.4	−5.2
750	736 ± 32	4.4	−1.8	777 ± 45	5.6	3.6
2500	2558 ± 97	3.7	2.3	2477 ± 139	5.6	−0.9
Valdecoxib						
5	4.9 ± 0.3	6.0	−0.6	4.6 ± 0.4	8.4	−8.1
100	100 ± 4.1	4.1	0.4	101 ± 5.1	5.1	−1.0
1000	999 ± 33	3.3	−0.1	1015 ± 27	2.7	1.6

at least three different concentrations (etoricoxib, 10, 750 and 2500 μg/L; valdecoxib, 5, 100 and 1000 μg/L) into each run. The results of the QC samples provided the basis for accepting or rejecting the series.

Figure 4 presents the plasma concentration time profiles in a male volunteer following oral administration of 90 mg etoricoxib or 20 mg valdecoxib.

DISCUSSION

The present study describes a new HPLC-MS^2-based method that represents a suitable approach to assess etoricoxib and valdecoxib concentrations in human plasma and should be easily extendable to other matrices.

A highly reliable precision and accuracy of the method within the validated concentration range could be demonstrated; no signal interferences from endogenous compounds were observed. Further quality controls revealed adequate stability of the analytes under all conditions applied.

In the positive ion mode the products resulting from the selected reaction could be explained by losses of the methyl sulphone group (etoricoxib) or the sulphonamide group (valdecoxib).

There are some published methods for determination of etoricoxib (Brautigam *et al.*, 2003; Rose *et al.*, 2002) or valdecoxib (Zhang *et al.*, 2003) in the literature. In contrast to our easy liquid–liquid extraction, all of these methods are based on a solid-phase extraction procedure. The formerly described methods used API triple quadrupole spectrometers (PE-Sciex) for the determination of COX 2 inhibitors. Only we used a Finnigan Mat LCQ ion trap spectrometer for the determination of etoricoxib and valdecoxib. Thus, the most analytical parameters are different from each other. Brautigam *et al.* (2003) used a turbo ion spray source working in positive ion mode; Rose *et al.* (2002) described a LC-MS-MS method using a heated nebulizer interface also operating in the positive ion mode. In both methods the respective mass transitions used for quantification of etoricoxib were 359 → 280, as in our method.

In the described LC-MS-MS method for valdecoxib in plasma with negative electrospray ionization (Zhang *et al.*, 2003), a main daughter different from our results was detected (313 → 118 vs 315 → 235). For our method we validated a range of 5–1000 µg/L (0.5–200 µg/L; Zhang *et al.*, 2003) that is usually needed for analysis of plasma samples from clinical studies (Fig. 4).

The validated method was tested to study the pharmacokinetic profile of etoricoxib and valdecoxib in one healthy male volunteer after administration of a normal single recommended dose (etoricoxib 90 mg or valdecoxib 20 mg). Analysis of plasma concentrations revealed that more than 90% of the AUC_∞ of both analytes was confined by the measured concentrations above the LOQ. Clearly, the sensitivity of our methods could well be enhanced. However, our results suggest that this is not necessary for analysis of clinical study.

The observed pharmacokinetic data (Fig. 4) are in accordance with the parameters published in the literature [etoricoxib 1 × 100 mg p.o.; t_{max} 1 h; c_{max} 1362 ± 333 µg/L; AUC_∞ 24.4 ± 8.8 µg h/L; $t_{1/2}$ 24.4 ± 8.8 h (Rodrigues *et al.*, 2003); valdecoxib, 10 mg oral steady state; t_{max} 2.25 h ± 0.71; c_{max} 161 ± 48 µg/L; AUC_∞ 1.48 ± 0.29 µg h/L; $t_{1/2}$ 8.1 ± 1.3 h (BEXTRA®, product information, Pfizer, July 2003)]. In conclusion, this new and easy analytical approach features a suitable method to assess the pharmacokinetic profile of the standard etoricoxib and valdecoxib regimen in humans.

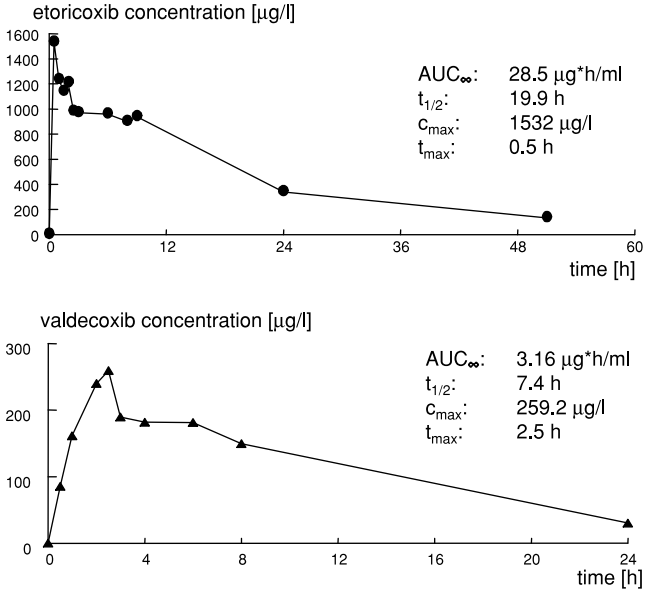

Figure 4. Plasma concentration time-profile of etoricoxib and valdecoxib (semilogarithmic plot) following oral administration of 90 or 20 mg monitored in a healthy human volunteer.

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