

HIGH-THROUGHPUT HPLC METHOD FOR RAPID QUANTIFICATION OF KETOPROFEN IN HUMAN PLASMA

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Manuscript received: May 2011

Abstract

A new high-throughput HPLC assay for the quantification of ketoprofen in human plasma was developed and validated. The separation was performed on a Zorbax SB-C18 column under isocratic conditions using a 55:45 (v/v) mixture of acetonitrile and 1% trifluoroacetic acid in water at 45°C with a flow rate of 1.5 mL/min. The detection of ketoprofen was performed at 257 nm. The human plasma samples (0.2 mL) were deproteinized with methanol (0.6 mL) and aliquots of 20 µL from supernatants obtained after centrifugation were directly injected into the chromatographic system. The method showed a good linearity ($r > 0.9997$), precision ($CV > 6.0\%$) and accuracy ($bias < 4.8\%$) over the studied range of 153.2 - 19155 ng/mL plasma. The lower limit of quantification (LLOQ) was 153 ng/mL and the recovery was between 96.5 - 103.6 %. The method is not expensive, it doesn't require a long time for plasma sample preparation and has a run-time of 2.1 min for instrument analysis (the retention time of ketoprofen was 1.7 min). The developed and validated high-throughput method is very simple, rapid and efficient, with wide applications in pharmacokinetics and bioequivalence studies.

Rezumat

S-a elaborat și s-a validat o metodă HPLC *high-throughput* pentru cuantificarea ketoprofenului în plasma umană. Separarea s-a realizat pe o coloană Zorbax SB-C18 în condiții izocratice folosind un amestec de acetonitril și acid trifluoroacetic 1% în apă (55:45, v/v) la 45°C cu un debit de 1,5 mL/min. Detectia ketoprofenului s-a efectuat la 257 nm. Probele de plasmă umană (0,2 mL) s-au deproteinizat cu metanol (0,6 mL) și din supernatantele obținute după centrifugare s-au injectat direct în sistemul cromatografic probe de 20 µL. Metoda a prezentat linearitate bună ($r > 0,9997$), precizie ($CV > 6,0\%$) și acuratețe ($bias < 4,8\%$) pe domeniul de concentrații studiat: 153,2 – 19155 ng/mL plasmă. Cea mai mică limită de cuantificare (LLOQ) a fost de 153 ng/mL și regăsirea între 96,5-103,6 %. Metoda nu este costisitoare, necesită un timp minim de pregătire a probelor de plasmă și un timp de analiză de 2,1 min pentru analiza instrumentală (timpul de retenție al ketoprofenului a fost de 1,7 min). Metoda *high-throughput* elaborată și validată este foarte simplă, rapidă și eficientă, cu largi aplicații în monitorizarea clinică a pacienților, dar și în studiile de farmacocinetică și bioechivalență.

Keywords: ketoprofen, human plasma, HPLC, high-throughput assay, therapeutic drug monitoring

Introduction

Ketoprofen, ($\pm$)-2-benzoylphenylpropionic acid, is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and antipyretic properties, effective and well tolerated in the treatment of various forms of rheumatic, traumatic and post-surgical pains [1]. It is readily absorbed after oral administration and it reaches the maximal plasma concentration within 1-2 h, but it requires frequent administrations to maintain therapeutic plasma levels, due to its short plasma elimination half-life (1-4 h) [2]. The therapeutic plasma

concentration can raise up to 20 µg/mL [3]. Ketoprofen's chemical structure contains a chiral atom. The cyclooxygenase inhibition activity has been mainly attributed to the S(+)-enantiomer, while the R(-)-enantiomer possesses analgesic properties independent of prostaglandin synthesis inhibition [4].

Various methods have been reported for enantioseparation of ketoprofen including high performance liquid chromatography (HPLC) [5-11], gas chromatography coupled with mass spectrometry [12, 13] or capillary zone electrophoresis [14]. However, these assays are complicated

as they involve the formation of diastereometric derivatives or the use of chiral columns.

In therapeutic drug monitoring, as well as in forensic and clinical toxicology, ketoprofen is usually quantified from human or animal plasma samples as a racemic, without enantioseparation. HPLC is the most frequently applied method [15-21].

The aim of this work was to develop and validate a new, simple and efficient high throughput HPLC method for the quantification of ketoprofen in human plasma for clinical concentration monitoring.

Materials and Methods

Reagents and materials

The reference standard of Ketoprofen was supplied as a gift by Terapia Ranbaxy SA (Cluj-Napoca, Romania). Acetonitrile of isocratic grade for liquid chromatography (LC), trifluoroacetic acid and methanol of analytical-reagent grade were purchased from Merck KGaA (Darmstadt, Germany). Bi-distilled, deionized water *pro injections* was purchased from Infusion Solution Laboratory of the University of Medicine and Pharmacy Cluj-Napoca (Romania). The human blank plasma was supplied by the Blood Transfusion Centre (Cluj-Napoca, Romania) from healthy volunteers, men and women.

Apparatus

The following apparatus were used: Analytical Plus and Precision Standard Balances (Mettler-Toledo, Switzerland); Vortex Genie 2 mixer (Scientific Industries, New York, USA); Ultrasonic bath Elma Transsonic 700/H (Singen, Germany). The used HPLC system was an 1100 series Agilent Technologies model (Darmstadt, Germany) consisting of a G1312A binary pump, an in-line G1379A degasser, an G1329A autosampler, a G1316A column thermostat and a G1314A UV Detector.

Chromatographic separation was performed on a Zorbax® SB-C18 (100 mm x 4.6 mm i.d., 3.5 µm) column (Agilent® Technologies, USA) under isocratic conditions, using a mobile phase of a 55:45 (v/v) mixture of acetonitrile and 1% (v/v) trifluoroacetic acid in water at 45°C with a flow rate of 1.5 mL/min. The detection of ketoprofen was performed at 257 nm using an UV Detector.

Chromatograms were processed using Quant Analysis® software (Agilent Technologies, USA).

Standard solutions

The stock solution of ketoprofen (1.27 mg/mL) was prepared by dissolving an appropriate amount of ketoprofen in methanol. The working solution (15.32 µg/mL) was prepared by appropriate dilution in drug-free human plasma. This solution was used to prepare plasma calibration standards with the concentrations of 153.2, 306.5, 613.0, 1225.9, 1532.4, 2298.6, 4597.2, 7662.0 and 19155.0 ng/mL,

respectively. Quality control (QC) samples of 459.7 ng/mL (lower), 1838.9 ng/mL (medium) and 6129.6 ng/mL (higher) were prepared by adding appropriate volumes of working solution to drug-free human plasma. The resultant plasma calibration standards and QC samples were pipetted into 15 mL polypropylene tubes and stored at -20°C until analysis.

Spiked samples for sample dilution validation

Solutions for sample dilution validation (VAL-DILconc - 38310 ng/mL) were freshly prepared on the day of analysis by adding 150 µL of stock solution to 4850 µL of blank plasma.

Sample preparation

Standards and plasma samples (0.2 mL) were deproteinized with methanol (0.6 mL). After vortex-mixture (10 s) and centrifugation (6 min at 6000 rpm), the supernatants (0.15 mL) were transferred into autosampler vials and 20 µL aliquots were then injected into the HPLC system.

Method validation

The specificity of the method was evaluated by comparing the chromatograms obtained from the plasma samples containing ketoprofen and those obtained from different plasma blank samples (n = 6). The concentration of ketoprofen was determined automatically by the instrument data system using peak areas and the external standard method. The calibration curve was determined with a linear regression analysis: $y = c + b \cdot x$, weighed linear response, where: x – concentration of the analyte (ng/mL), y – peak area of the analyte, b – slope of the calibration curve, c – intercept of the calibration curve.

The intra-day precision (expressed as coefficient of variation, CV %) and accuracy (expressed as relative difference between obtained and theoretical concentration, bias %) were determined by analysing five different samples (n = 5) from each QC sample (at lower, medium and higher levels) on the same day. The inter-day precision and accuracy were determined by analysing one QC sample from each level (low, medium and high) on five different days (n = 5).

The lower limit of quantification (LLOQ) was established as the lowest calibration standard with an accuracy and precision less than 20%.

The absolute recoveries were measured by comparing the response of the spiked plasma with the response of standards in solvent having the same concentration of ketoprofen as the plasma.

For sample dilution validation, one sample of VAL-DILconc was diluted 1:10 (v/v) with blank plasma and analysed during inter-run assays, on five different days (n = 5). At least in one day, five samples of VAL-DILconc (n = 5) were further

diluted 1:10 (v/v) and analysed in order to assess within-day precision and accuracy.

The ketoprofen stability in plasma at lower and higher levels ($n = 5$) was investigated. For the short room-temperature stability (RTS) study, the samples were prepared and kept at room temperature for 4 h, then they were processed and analysed by HPLC. For the post-preparative stability (PPS) study, the samples were prepared, processed and thermostatted at 25°C in the HPLC autosampler for 12-24 h before the chromatographic assay. For the long-term stability (LTS) study, the samples were stored below -20°C and analysed during 2 months. For the freeze-thaw stability (FTS) study, the samples were subjected to three cycles of freeze-thaw operations in three consecutive days ($n = 3$). The requirement for the stability of the drug is that the difference between mean concentrations of the tested samples in various conditions and nominal concentrations to fall within $\pm 15\%$ range.

Results and Discussion

We propose a very simple and rapid pre-treatment of plasma samples that includes protein precipitation

(PP) with methanol, centrifugation and direct LC injection of supernatant aliquots.

Several researchers prefer to include intermediate purification steps in the plasma samples preparation process, like liquid-liquid extraction (LLE) [16, 19] or solid phase extraction (SPE) [17, 18] in order to increase sensitivity (Table I). These steps, however, increase the analysis time as well as the costs, and can affect the recovery [22]. Therefore, Roda et al. [16] reported a LC-UV method using LLE in diethyl-ether and obtained a good LLOQ of 0.05 $\mu\text{g/mL}$. Suenami et al. [18] published a LC-MS method using on-line SPE and they obtained a very good LLOQ of 0.025 $\mu\text{g/mL}$, but the average recovery yield was only 83.8 %.

Other researchers prefer the PP with methanol [15, 21] or acetonitrile [20] as a pre-treatment for plasma samples (Table I). Martin et al. [15] determined ketoprofen from human plasma by LC-UV after PP with methanol, obtaining a LLOQ of 3.1 $\mu\text{g/mL}$ and a recovery of 98.2%. Granero et al. [20] reported a LC-UV method using PP with acetonitrile, having a LLOQ of 0.71 $\mu\text{g/mL}$. Our LC-UV method is better than other similar ones in terms of sensitivity and recoveries: our LLOQ was 0.153 $\mu\text{g/mL}$ and the recovery was between 96.5 - 103.6 %.

Table I

Analytical characteristics of reported HPLC methods for the determination of ketoprofen in human or animal plasma samples

References	Matrix	Detection mode ^a	Mobile phase constituents ^b	Pre-treatment/extraction ^c	LOQ ^d ($\mu\text{g/mL}$)	Rt ^e (min)
15	Human plasma	UV (254 nm)	A: aqueous formic acid / formate buffer (0.1M), pH 3; B: MeOH, gradient	PP	3.1	14.3
16	Human plasma	UV (254 nm)	ACN : 0.01 M KH_2PO_4 , pH 3.5 (40:60, v/v), isocratic	LLE	0.05	NA
17	Human plasma	MS-ESI, negative ion mode	ACN: 10 mM ammonium formate in water, pH 3.5 (50:50, v/v), isocratic	SPE	0.1	1.8
18	Human plasma	MS-ESI, negative ion mode	10mM ammonium formate aqueous solution (pH 3.25) :CAN (40:60, v/v)	PP, On-line SPE	0.025	4.4
19	Dog plasma	UV (260 nm)	0.05 M pH 7.0 phosphate buffer : ACN (84:16, v/v), isocratic	LLE	5	NA
20	Dog plasma	UV (258 nm)	ACN : water, adjusted at pH 3.02 (35:65, v/v)	PP	0.71	~17
21	Mice plasma	UV (254 nm)	MeOH : 0.02 M KH_2PO_4 , pH 3.5 (70:30, v/v), isocratic	PP	NA	NA

^aESI, electrospray ionization; ^bACN, acetonitrile; MeOH, methanol; ^cSPE, solid-phase extraction; LLE, liquid-liquid extraction; PP – protein precipitation; ^dLOQ, limit of quantification; ^eRT, retention time; NA, not available.

As the therapeutic plasma levels of ketoprofen are as low as 20 $\mu\text{g/mL}$ [3], the LLOQ established in our method can be accepted in routine procedures for therapeutic level monitoring of ketoprofen in adult and children human plasma.

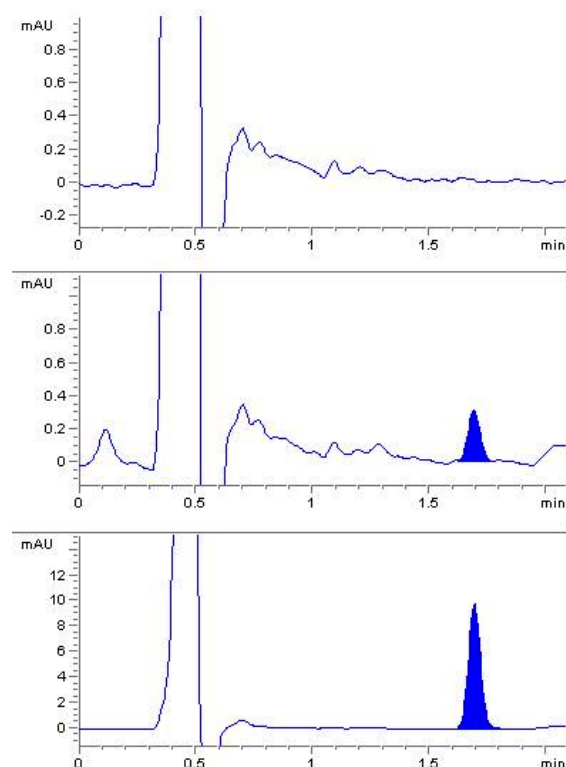
HPLC assay

The chromatographic conditions, especially the composition of mobile phase, were optimized in several trials to achieve good signal, short retention

time of ketoprofen and, consequently, high-throughput analysis. The best results were obtained with the mixture of acetonitrile and 1% trifluoroacetic acid in water (55:45, v/v) under isocratic conditions. In the selected chromatographic conditions, the retention time of ketoprofen was 1.7 min and the analytical run-time was 2.1 min. Other LC published methods for quantitation of ketoprofen in human or animal plasma have longer run-times,

with retention times of ketoprofen more than 4.4 min (Table 1) [15, 18, 20]. Only Suenami et al. [17] reported a LC-MS method coupled with SPE extraction of the samples having a retention time of

1.8 min for ketoprofen, but they have developed the method for rapid and simultaneous determination of several NSAIDs in human plasma with a run-time of 8 min.



Method validation

The method was validated in accordance with international regulations [23-28]. Representative chromatograms of drug-free plasma and plasma spiked with ketoprofen at the LLOQ are shown in Figure 1. No interfering peaks from the endogenous plasma components were observed at and around the retention time of ketoprofen.

The calibration curves were linear over the concentration range of 153.2 – 19155 ng/mL in human plasma, with a correlation coefficient greater than 0.9997. The LLOQ was 153 ng/mL. The values obtained during the plasma samples validation process (for intra-day and inter-day precision and accuracy tests) are shown in Table II and Table III, respectively.

Figure 1.

Representative chromatograms of drug-free plasma (upper image), plasma spiked with ketoprofen at the lower limit of quantification (153 ng/mL) (middle) and plasma sample obtained from a patient 2 h after the administration of 50 mg ketoprofen (concentration found: 4428 ng/mL) (lower image)

Table II

The intra-day precision (CV %) and accuracy (bias %) and recovery data for the measurement of ketoprofen in human plasma (n = 5)

Nominal concentration (ng/mL)	Found concentration mean		CV (%)	Bias (%)	Recovery	
	ng/mL	± SD			(%)	± SD
153.24	152.44	3.57	2.3	-0.5	101.1	2.1
459.72	441.58	17.47	4.0	-3.9	96.5	3.9
1838.88	1786.41	23.68	1.3	-2.9	100.3	1.3
6129.60	5932.74	28.72	0.5	-3.2	99.2	0.5

Table III

The inter-day precision (CV %) and accuracy (bias %) and recovery data for the measurement of ketoprofen in human plasma (n = 5)

Nominal concentration (ng/mL)	Found concentration mean		CV (%)	Bias (%)	Recovery	
	ng/mL	± SD			(%)	± SD
153.24	160.57	9.61	6.0	4.8	103.6	3.5
459.72	444.83	5.13	1.2	-3.2	97.9	0.7
1838.88	1780.79	19.84	1.1	-3.2	99.6	1.5
6129.60	5957.78	88.27	1.5	-2.8	99.2	1.5

All values for accuracy and precision were within recommended limits. The recovery values were between 96.5 - 103.6 %. Plasma dilutions could be

made with a CV% less than 1.8 % and an accuracy less than 2.6 % for within-run and between-run determinations (Table IV).

Table IV

The precision (CV %) and accuracy (bias %) and recovery data for the dilution validation (n = 5)

	Nominal concentration (ng/mL)	Found concentration mean		CV (%)	Bias (%)
		ng/mL	± SD		
Intra-day	3831.0	3889.4	69.3	1.8	1.5
Inter-day	3831.0	3932.3	29.3	0.7	2.6

Ketoprofen showed a good stability in plasma samples at room temperature for 4 h (CV of -5.0 % at lower level and -3.5 % at the higher level, respectively) and a good post-preparative stability in autosampler for at least 17 hours at 25°C before the chromatographic assay (CV of -3.2 % at lower level and -2.9 % at higher level, respectively). It has a good long-term stability in plasma stored below -20°C for 5 weeks (CV of -2.1 % at lower level and 0.1 % at higher level, respectively) and a good freeze-thaw stability in plasma submitted to three freeze-thaw cycles (CV of -7.5 % at lower level and -2.2 % at higher level, respectively).

Method application

The newly developed and validated analytical method was applied in a pharmacokinetic study of ketoprofen in healthy volunteers. A representative chromatogram of a plasma sample obtained from a healthy volunteer, 2 h after the administration of ketoprofen, is shown in Figure 1 (found concentration: 4428 ng/mL).

Conclusions

Our developed LC-UV method is simple, rapid, accurate and not expensive. In comparison with other published HPLC assays for the quantitation of ketoprofen in human or animal plasma, our method performs better in terms of speed and costs, which are essential attributes for methods used in routine analysis. The method was validated over the concentration range of 153.2 – 19155 ng/mL, covering therapeutic (< 20 µg/mL) plasma levels of ketoprofen. This high-throughput method was successfully applied in a pharmacokinetic study, but it can have wide applications in therapeutic concentration monitoring, drug-interaction and toxicological studies of ketoprofen, too.

References

1. Sarzi-Putini P., Atzeni F., Lanata L., Bagnasco M., Colombo M., Fischer F., D'Imporzano M., Pain and ketoprofen: what is its role in clinical practice? *Reumatismo*, 2010; 62(3): 172-188.
2. Moffat A.C., Osselton M.D., Widdop B., Clarke's Analysis of Drugs and Poisons in pharmaceuticals, body fluids and postmortem material. vol. 2. 3rd ed. Pharmaceutical Press, London, 2004; 1156-1157.
3. Flanagan R.J., Guidelines for the interpretation of analytical toxicology results and unit of measurement conversion factors. *Annals of Clinical Biochemistry*, 1998; 35: 261-267.
4. Ossipov M.H., Jerussi T.P., Ren K., Sun H., Porreca F., Differential effects of spinal (R)-ketoprofen and (S)-ketoprofen against signs of neuropathic pain and tonic nociception: evidence for a novel mechanism of action of (R)-ketoprofen against tactile allodynia. *Pain*, 2000; 87(2): 193-199.
5. Lovlin R., Vakily M., Jamali F., Rapid, sensitive and direct chiral high-performance liquid chromatographic method for ketoprofen enantiomers. *J. Chromatogr. B Biomed. Appl.*, 1996; 679(1-2): 196-198.
6. Boisvert J., Caillé G., McGilveray I.J., Qureshi S.A., Quantification of ketoprofen enantiomers in human plasma based on solid-phase extraction and enantioselective column chromatography. *J. Chromatogr. B Biomed. Sci. Appl.*, 1997; 690(1-2): 189-193.
7. Baeyens W.R., Van der Weken G., Hastraete J., Aboul-Enein H.Y., Corveleyn S., Remon J.P., García-Campaña A.M., Deprez P., Application of the restricted-access precolumn packing material alkyl-diol silica in a column-switching system for the determination of ketoprofen enantiomers in horse plasma. *J. Chromatogr. A.*, 2000; 871(1-2): 153-161.
8. Lees P., Taylor P.M., Landoni F.M., Arifah A.K., Waters C., Ketoprofen in the cat: pharmacodynamics and chiral pharmacokinetics. *Vet. J.*, 2003; 165(1): 21-35.
9. Igarza L., Soraci A., Auza N., Zeballos H., Some pharmacokinetic parameters of R-(-)- and S-(+)-ketoprofen: the influence of age and differing physiological status in dairy cattle. *Vet. Res. Commun.*, 2004; 28(1): 81-87.
10. Montoya L., Ambros L., Kreil V., Bonafine R., Albarellos G., Hallu R., Soraci A., A pharmacokinetic comparison of meloxicam and ketoprofen following oral administration to healthy dogs. *Vet. Res. Commun.*, 2004; 28(5): 415-428.
11. Pavalache G., Dorneanu V., Validation of an analytical method based on high performance liquid chromatography for determination of loratadine in pharmaceutical preparations and biological environments. *Farmacia*, 2011; 59(2): 200-208.
12. Jack D.S., Rumble R.H., Davies N.W., Francis H.W., Enantiospecific gas chromatographic-mass spectrometric procedure for the determination of ketoprofen and ibuprofen in synovial fluid and plasma: application to protein binding studies. *J. Chromatogr.*, 1992; 584(2): 189-197.
13. Paik M.J., Nguyen D.T., Kim K.R., Enantioseparation of flurbiprofen and ketoprofen in patches and in urine excretions by achiral gas chromatography. *Arch. Pharm. Res.*, 2004; 27(12): 1295-1301.
14. Głowka F.K., Karazniewicz-Lada M., Determination of Ketoprofen Enantiomers in Clinical Samples of Plasma, Synovial Fluid and Urine. *Chromatographia*, 2008; 67: S97-S105.
15. Martin M.J., Pablos F., Gonzalez A.G., Simultaneous determination of caffeine and non-steroidal anti-inflammatory drugs in pharmaceutical formulations and blood plasma by reversed-phase HPLC from linear gradient elution. *Talanta*, 1999; 49: 453-459.
16. Roda A., Sabatini L., Mirasoli M., Baraldini M., Roda E., Bioavailability of a new ketoprofen formulation for once-daily oral administration. *Int. J. Pharm.*, 2002; 241(1): 165-172.
17. Suenami K., Lim L.W., Takeuchi T., Sasajima Y., Sato K., Takekoshi Y., Kanno S. Rapid and

- simultaneous determination of nonsteroidal anti-inflammatory drugs in human plasma by LC-MS with solid-phase extraction. *Anal Bioanal. Chem.*, 2006; 384(7-8): 1501-1505.
18. Suenami K., Lim L.W., Takeuchi T., Sasajima Y., Sato K., Takekoshi Y., Kanno S., On-line sample extraction and enrichment of non-steroidal anti-inflammatory drugs by pre-column in capillary liquid chromatography mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2007; 846(1-2): 1776-1783.
 19. Vergote G.J., Vervaeet C., Van Driessche I., Hoste S., De Smedt S., Demeester J., Jain R.A., Ruddy S., Remon J.P., *In vivo* evaluation of matrix pellets containing nanocrystalline ketoprofen. *Int. J. Pharm.*, 2002; 240(1-2): 79-84.
 20. Granero G.E., Amidon G.L., Possibility of enterohepatic recycling of ketoprofen in dogs. *Int. J. Pharm.*, 2008; 349(1-2): 166-171.
 21. Qiu H.X., Liu J., Kong H., Liu Y., Mei X.G., Isobolographic analysis of the antinociceptive interactions between ketoprofen and paracetamol. *Eur. J. Pharmacol.*, 2007; 557(2-3): 141-146.
 22. Aramă C., Varvara A., Monciu C.M., Development and validation of a new capillary zone electrophoresis method for the assay of ondansetron. *Farmacia*, 2011; 59(1): 34-43.
 23. Guidance for Industry, Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Federal Register, 2001; 66.
 24. Guidance on the Investigation of Bioavailability and Bioequivalence. The European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products, 2001; CPMP/EWP/QWP/1401/98.
 25. Vlase L., Popa D.S., Muntean D., Miha D., Leucuta S., A New, High-Throughput HPLC/MS Assay for Therapeutic Level Monitoring of Digoxin in Human Plasma. *J. AOAC Intern.*, 2009; 92(5): 1390-1395.
 26. Butnariu A., Popa D.S., Vlase L., Andreica M., Muntean D., Leucuta S., New high-throughput liquid chromatographic tandem mass spectrometry assay for therapeutic drug monitoring of carvedilol in children with congestive heart failure. *RRML*, 2009; 15(2): 7-15.
 27. Vlase L., Popa D.S., Leucuța S.E., Loghin F., Bioanalysis of methadone in human plasma and urine by LC/MS/MS. *Rev. Roum. Chimie*, 2008; 53(12): 1157-1164.
 28. Vlase L., Popa D.S., Muntean D., Achim M., A New LC/MS/MS Method for Determination of Lisinopril in Human Plasma. *Studia Universitatis Babeș-Bolyai Chemia*, 2010; 55(1): 91-101.