

DEVELOPMENT AND VALIDATION OF A NEW CAPILLARY ZONE ELECTROPHORESIS METHOD FOR THE ASSAY OF ONDANSETRON

CORINA ARAMĂ¹, ANDREEA VARVARA^{2*}, CRINA - MARIA MONCIU¹

¹ *Analytical Chemistry Department, Faculty of Pharmacy, UMF Carol Davila, Traian Vuia Street no. 6, Bucharest, Romania*

² *Quality of Medicines Control Department, National Medicines Agency, Av. Sanatescu Street no. 48, Bucharest, Romania*

**corresponding author: andreea.varvara@anm.ro*

Abstract

An effective new capillary zone electrophoresis (CZE) assay has been developed for the determination of ondansetron hydrochloride and one of its impurities, ((3*RS*)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one) noted impurity A. Best resolution between the two analytes was achieved using a 35 mM phosphate buffer (pH 6.0) as running electrolyte, 20 kV applied voltage at 15°C, with direct UV detection at 216 nm; the migration times were 3.05 minutes and 3.3 minutes, respectively. The newly established method was validated with respect to linearity, precision, accuracy, selectivity, detection and quantitation limits.

The proposed CZE method is simple, fast and reliable, with convenient results in the assay of ondansetron and its impurity in pharmaceutical dosage forms (injectable solutions).

Rezumat

A fost dezvoltată o metodă electroforetică (electroforeză capilară zonală) eficientă în identificare și dozarea clorhidratului de ondansetron și a uneia dintre impuritățile acestuia, ((3*RS*)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol -4-one) notată impuritatea A. Rezoluția optimă între cei doi analiți s-a obținut folosind ca electrolit de lucru o soluție tampon fosfat 35 mM (pH 6,0), un potențial aplicat de 20 kV la 15°C, cu detecție directă UV la 216 nm. Timpii de migrare caracteristici sunt 3,05 minute pentru ondansetron și, respectiv, 3,3 minute pentru impuritatea A. Metoda nou instituită a fost validată pentru parametrii: liniaritate, precizie, acuratețe, selectivitate și limite de detecție și cuantificare.

Metoda de electroforeză capilară zonală propusă este simplă, rapidă și eficientă, cu rezultate bune la determinarea ondansetronului și a impurității sale din forme farmaceutice (soluții injectabile).

Keywords: Ondansetron hydrochloride assay, Capillary zone electrophoresis

Introduction

Ondansetron (figure 1), a selective serotonin 5-HT₃ receptor antagonist is a highly used drug in patients with nausea and vomiting

associated with cancer chemotherapy, radiotherapy or anesthesia and surgery [6].

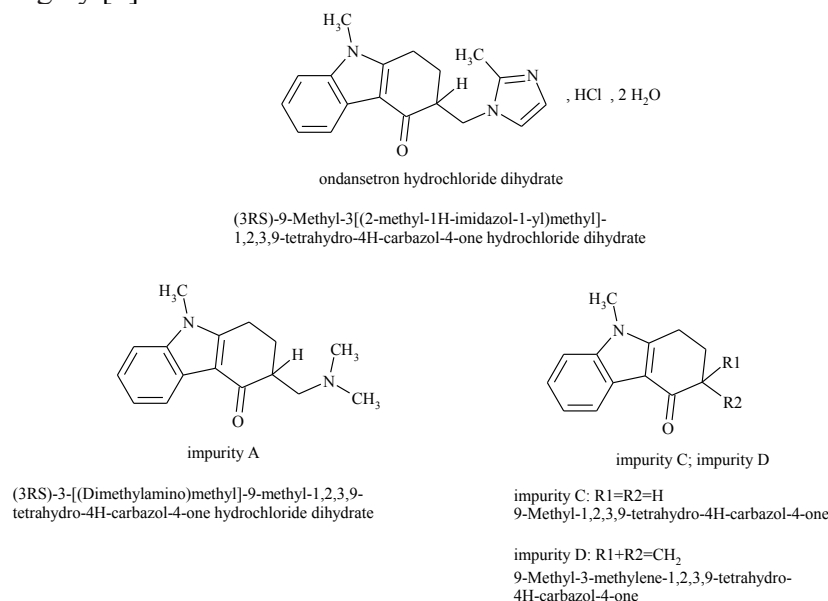


Figure 1.

Structure of ondansetron hydrochloride dihydrate and its synthesis impurities

Due to the marked polarity of the molecule and the relatively low water solubility, reported methods for the assay of ondansetron are, mostly, reversed-phase HPLC using nitrile, octylsilyl and phenyl silica stationary phases and mixtures of polar solvents as mobile phases [1, 6]. New HPLC methods using stationary phases with different retention mechanisms [8,9,11] have been presented recently for the assay of ondansetron and/or its synthesis impurities (fig. 1). There are reported, also, ion-pair chromatography (IPC) assay methods allowing its determination in bulk and pharmaceutical dosage forms [10], as well as in complex mixtures with ranitidine and paclitaxel, or piperaciline and tazobactam [3, 4].

Several high performance capillary zone electrophoresis methods are presented for the enantiomeric separation of ondansetron or for its assay in injection [5, 7, 12].

Capillary zone electrophoresis (CZE) is a very convenient alternative method for the separation and analysis of pharmaceuticals (mostly, for polar, easily ionizable ones), as its efficiency is similar to that of HPLC, using less sample and more affordable reagents. The aim of this

work was to investigate the specific possibilities to use CZE in the assay of ondansetron (a weak basic compound ($pK_a=7.4$) undergoing ionization in solutions with pH below 7.0) and its synthesis impurities.

Materials and methods

Chemicals

Analytical grade citric acid, KH_2PO_4 , K_2HPO_4 , NaOH and HCl were purchased from Merk KgA, Germany. Citrate buffer 60 and 35 mM (pH 3, 4 and 5) and phosphate buffer 60 and 35 mM (pH 6 and 7) were prepared using ultrapure water for chromatography [13]; the pH was determined potentiometrically.

Ondansetron hydrochloride dihydrate and impurities A, C and D working standards were provided by Dr. Reddy's (India). The water content of ondansetron hydrochloride dihydrate was 10.0% (determined by Karl-Fischer titration).

Ondansetron hydrochloride dihydrate active substance was provided by Dr. Reddy's (India). The water content was 9.9% (determined by Karl Fischer titration).

Osetron[®] 4mg/2mL, injectable solution (i.v.) - Dr. Reddy's, India - was selected as pharmaceutical dosage form.

Standard solution of ondansetron hydrochloride was prepared by dissolving 10.0mg ondansetron hydrochloride working standard in 2mL methanol, then completing the solution to 25.0mL using pH=6 phosphate buffer. Standard solutions of different concentrations used in validation were prepared by appropriate dilution, using pH=6 phosphate buffer (10-300 $\mu\text{g/mL}$). Added methanol was intended to enhance ondansetron solubility and acted also as an electroosmotic flow marker.

Sample solutions were obtained by diluting 0.4mL injectable solution to 10.0mL with phosphate pH=6.0 buffer.

All solutions were filtered through a 0.22 μm microfilter prior use.

Electrophoretic system and conditions

CZE was performed using an Agilent Technologies G1602A equipment with an on-column diode-array detector. *Agilent Chemstation* ver. B.0 2.0x soft-ware was used for data acquisition. Fused-silica capillaries of 48.5 cm (40 cm effective length) x 50 μm ID were used.

Whenever it was necessary, ultrapure water for chromatography was prepared using a Barnstead EASYpure Rodi equipment.

Electrophoretic separation was performed using as running buffer a 35 mM phosphate solution pH 6.0. Prior each sequence of analysis the capillary was rinsed 5 min with 1M NaOH, 10 min with water and 10 min with the running buffer. At the end of the day, the capillary was washed for 10 min with water, 2 min with 1M NaOH, 5 min with water and flushed with air for 2 min.

The temperature of the cassette was 15°C and the applied voltage, 20 kV. The samples were introduced through hydrodynamic injection (5 sec at 50 mbar).

Analytes were detected by UV absorbance at 216 nm (full spectra recorded between 200 and 400 nm).

Triplicate injections were performed and peak areas were used for the quantification.

Results and discussion

In the preliminary tests, we have tried to resolve the complex mixture of ondansetron, impurity A, impurity C and impurity D.

Due to the non-ionizable structure of impurities C and D, their mobility was due only to the electroosmotic flow in the column all over the pH range investigated (fig. 2).

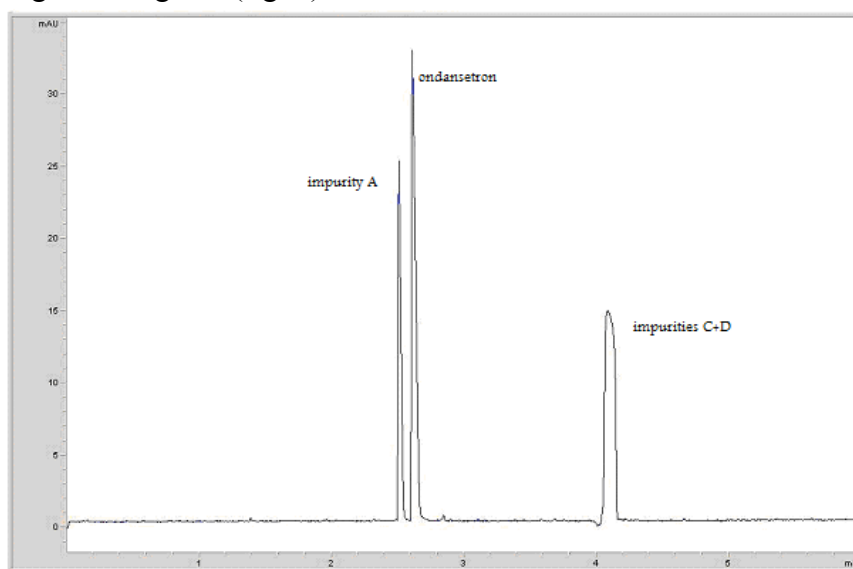


Figure 2.

Electropherogram of ondansetron and impurities A, C and D using a 35 mM citrate buffer pH 3.5 as running electrolyte

Further in our study we focused on establishing the optimum experimental conditions for the simultaneous assay of ondansetron and impurity A in mixtures. We tested the effect of the applied potential, the temperature and the pH of the running buffer on the effective electrophoretic mobility of ondansetron and on the efficiency of the separation achieved.

Effect of the pH of the running buffer

Several citrate and phosphate buffers with 35 and 60 mM concentrations and pH values between 3.0 - 7.0 were tested. From our previous studies [3-6], the separation of impurity A and ondansetron is critical in HPLC. The most convenient electrophoretic separation, with a resolution of 15, was obtained when using a 35 mM phosphate buffer pH 6.0 as running electrolyte (fig. 3).

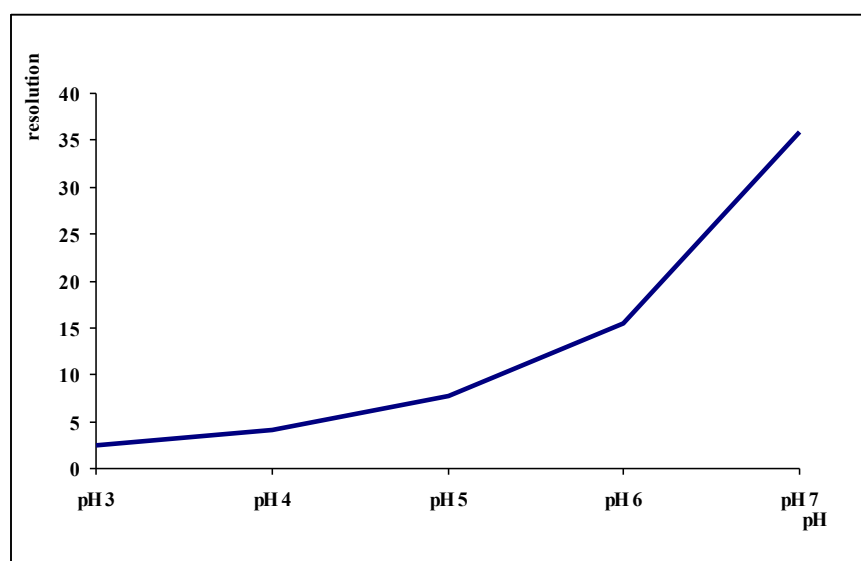


Figure 3.

Resolution profile in the 3.0 -7.0 pH range

Effect of the temperature

The temperature effect on the migration rate and on the effective electrophoretic mobility was tested between 15-30°C. The migration rate and also the effective electrophoretic mobility decreased at a high temperature value. A temperature of 15°C was chosen for the determinations (fig. 4).

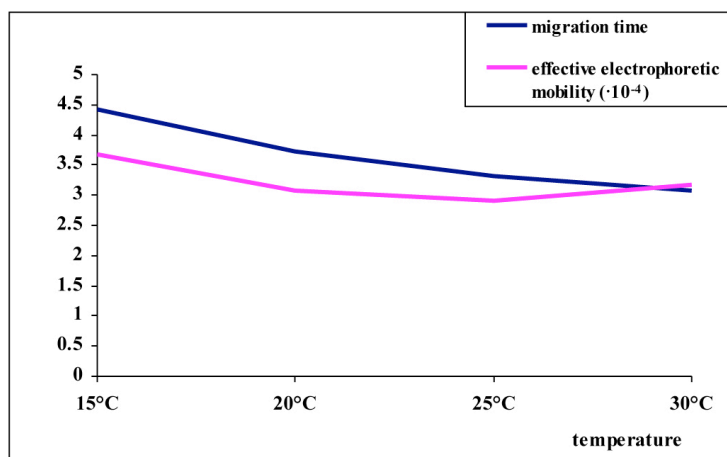


Figure 4.

Migration time (min) and effective electrophoretic mobility $\text{cm}^2/(\text{V}\cdot\text{s})$ variation with the temperature in the 15-30°C range

Effect of the applied voltage

The effect of the applied voltage was investigated from 15 to 30 kV. The best resolution and a convenient value of the current (cca. 45 μA) were obtained for 20 kV (fig. 5).

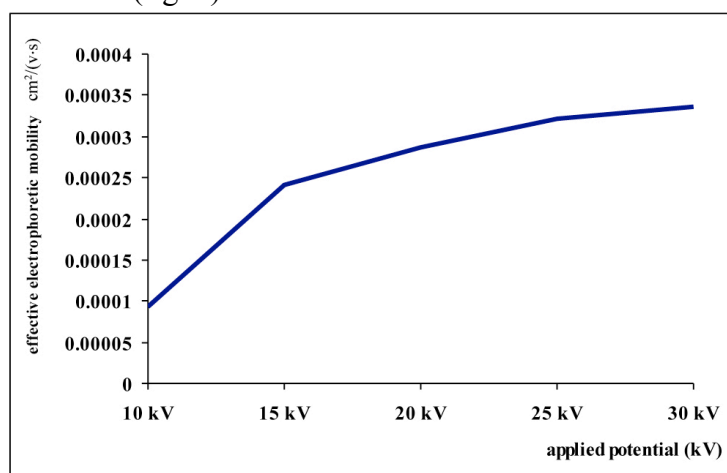
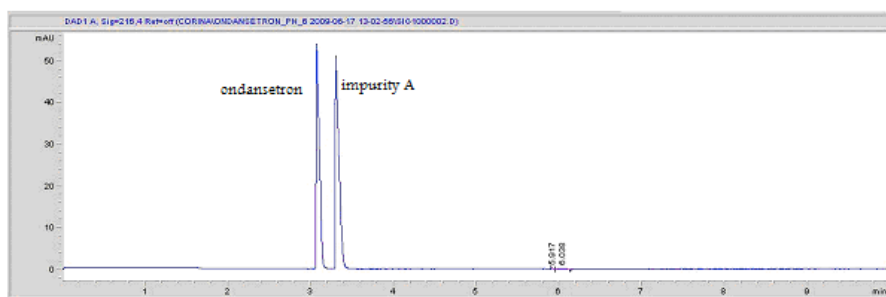


Figure 5.

Effective electrophoretic mobility variation with the applied voltage in the 10-30kV range

Using the selected parameters for the assay of ondansetron hydrochloride using capillary zone electrophoresis, we obtained an efficient electrophoretic separation, with symmetric peaks at 3.05 minutes and 3.3 minutes, for ondansetron and its impurity A, respectively (fig. 6).

**Figure 6**

Electropherogram of ondansetron hydrochloride and impurity A
(running buffer 35 mM phosphate buffer pH 6.0, separating voltage 20 kV, cassette temperature 15°C, hydrodynamic injection 5 s at 50 mbarr, UV detection wavelength 216 nm)

Validation of CZE assay procedure

The analytical procedure was validated according to ICH requirements (ICH – The International Conference on harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) [2], with respect to stability, specificity, linearity, detection and quantification limits, range, accuracy and precision. Validation data is summarized in table I.

Table I
Summary of the validation data obtained

Validation criterion	Parameter	Results	
		Ondansetron	Impurity A
Linearity	Regression parameters	$Y = 0.0022X - 0.0034$	$Y = 0.0027X + 0.0189$
	Correlation coefficient	0.9992	0.9998
Precision	Repeatability*	RSD% = 2.43 (peak area: 65.4 ± 1.09)	RSD% = 1.85 (peak area: 68.7 ± 1.22)
		RSD% = 2.21 (migration time: $3.05\text{min} \pm 0.05$)	RSD% = 2.16 (migration time: $3.31\text{min} \pm 0.07$)
	Intermediate precision**	RSD% = 0.72	RSD% = 1.28
Accuracy		$100.33 \pm 1.54\%$ RSD% = 0.62 (n = 6)	$100.71 \pm 0.92\%$ RSD% = 0.83 (n = 6)
Recovery***		$100.58 \pm 0.29\%$ RSD% = 0.68	$100.12 \pm 1.59\%$ RSD% = 0.64
Detection limit (signal-to-noise method)		1.5 µg/mL (RSD% = 0.92, n = 6)	1.3 mg/mL (RSD% = 0.87, n = 6)
Quantification limit (signal-to-noise method)		4 µg/mL (RSD% = 0.43, n = 6)	3.9 mg/mL (RSD% = 0.52, n = 6)

*10 consecutive injections of ondansetron (50µg/mL)

**3 different concentrations, in triplicate, in 3 consecutive days

***3 samples, in triplicate, for 3 concentration levels (80-120%)

The calibration chart was linear in the concentration range 10.24 - 307.2 $\mu\text{g/mL}$ ondansetron hydrochloride dihydrate and 6.4 - 64 $\mu\text{g/mL}$ impurity A.

The results showed a good correlation between the response and the concentration. The response factor of the UV detector is quite constant.

The method proved to be selective against the running buffer and the excipients for injectable solution (citric acid monohydrate, sodium citrate and sodium chloride) (fig. 7). A mixture of the injection excipients, before and after being spiked with standard and the commercial solution for injection were tested and no interfering peaks were present.

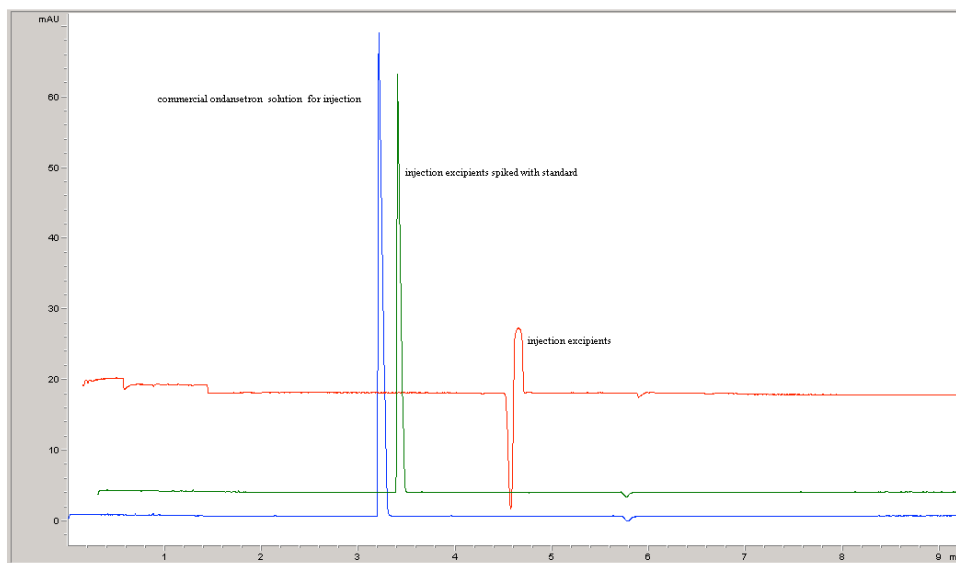


Figure 7

The electropherograms of injection excipients, injection excipients spiked with standard and commercial ondansetron solution for injection (running buffer 35 mM phosphate buffer pH 6.0, separating voltage 20 kV, cassette temperature 15°C, hydrodynamic injection 5 s at 50 mbarr, UV detection wavelength 216 nm, ondansetron 50 $\mu\text{g/mL}$)

Assay of ondansetron in bulk and pharmaceutical dosage forms

Ondansetron was quantified both from bulk and injections.

The impurity was determined in ondansetron standard solutions spiked with impurity A. The assay was possible only for samples in which the impurity level was more than 0.6% in ondansetron (this level is higher

than the limit recommended in the European Pharmacopoeia monograph).

The results obtained in the assay of ondansetron hydrochloride dihydrate, both in bulk and pharmaceutical dosage forms, are reported in table II.

Table II

Results of the assay
of ondansetron hydrochloride active substance and dosage forms

Sample type	Assay results (%)						Average %	RSD %	Confidence interval (n=6, P=95%)
Active substance	99.67	100.56	98.98	100.70	100.31	1100.29	100.08	0.64	99.41 – 100.76 % (as ondansetron hydrochloride)
Osetron®	1100.18	1100.81	999.44	1100.42	1100.41	1100.16	100.24	0.45	99.76 – 100.72 % (1.99– 2.01 mg/mL) as ondansetron)

Conclusions

The simple and rapid CZE method developed may be an alternative to reversed phase – high performance liquid chromatography methods (RP-HPLC), whenever accurate determination of ondansetron hydrochloride is needed.

The newly established method was thoroughly validated, in order to prove its suitability for the assay of ondansetron hydrochloride in bulk and pharmaceutical dosage forms.

Impurities C and D arising from the synthesis of ondansetron could not be quantified using capillary zone electrophoresis, as they are mostly neutral in a pH range between 3.0 and 7.0.

The assay of the impurity A is possible only in samples containing more than 0.6% analyte.

References

1. XXX, the European Pharmacopoeia 6th Edition, Strasbourg, 2008;
2. XXX, Validation of Analytical Procedures: Text and Methodology Q2(R1), ICH Harmonised Tripartite Guideline, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, November 2005
3. Burm J-P., Jhee, S.S., Moon Z.S.K., Jeong E., Nii L., Fox J.L., Gill M.A., Stability of paclitaxel with ondansetron hydrochloride or ranitidine hydrochloride during simulated Y-site administration, *Am. J. Health-Syst.Pharm.* 1994, 51, 1201-1204
4. Chung K.C., Moon Y.S.K., Ulrich R.W., Gill M.A., Compatibility of ondansetron

- hydrochloride and piperacillin sodium tazobactam sodium during simulated Y-site administration, *Am. J. Health-Syst.Pharm.* 1995, 52, 1554-1556
5. Li, H.; Shi, W.; You, H., Determination of ondansetron in injection by high performance capillary zone electrophoresis, *Chinese Journal of Chromatography* 1998, 16(3), 250-251
 6. Salem I.I., Lopez Ramos J.M., Galan Cerezo A., Ondansetron hydrochloride, *Anal. Profiles Drug Subst. Excipients* 2001, 27, 301-338
 7. Siluveru M, Stewart J.T., Enantioselective determination of *S*-(+)- and *R*-(-)-ondansetron in human serum using derivatized cyclodextrin-modified capillary electrophoresis and solid-phase extraction, *Journal of Chromatography B: Biomedical Sciences and Applications* 1997, 691 (1), 217-222
 8. Varvara A., Monciu C-M., Aramă C., Popescu C., A new stationary phase in the liquid chromatographic assay of ondansetron hydrochloride, *Farmacia*, 2007, 55 (2), 121-131
 9. Varvara A., Monciu C-M., Aramă C., Popescu C., The HPLC assay of ondansetron hydrochloride and its impurities using a new stationary phase, *Farmacia*, 2008, 56 (2), 154-165
 10. Varvara A., Monciu C-M., Aramă C., Popescu C., Ion-pair reversed-phase high performance liquid chromatography of ondansetron hydrochloride using sodium heptanesulphonate as a counterion, *Farmacia*, 2009, 57 (4), 442-451
 11. Varvara A., Monciu C-M., Aramă C., Popescu C., Application of selective bonded phase in the liquid chromatographic assay of ondansetron hydrochloride and its impurities, *Farmacia*, 2009, 57 (5), 570-581, <http://www.sciencedirect.com/science/journal/15700232>
 12. Zhou, Y.; Feng F., Zhang Z.-X., Enantioselective determination of two enantiomers of ondansetron in rat plasma by HPCE and their stereoselective pharmacokinetic study in rats, *Chinese Pharmaceutical Journal* 2006, 41 (7), 541-544
 13. www.raell.daemon.co.uk/chem/logp/logppka.html, accessed: November 14th 2008

Manuscript received: April 22nd 2010