ORIGINAL ARTICLE

DIRECT QUANTIFICATION OF MELOXICAM FROM TRANSDERMAL THERAPEUTIC SYSTEMS BY NIR SPECTROSCOPY

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Abstract

This study describes the development and validation of a new near infrared (NIR) spectroscopic method for the determination of meloxicam content from transdermal therapeutic systems (TTS). For this purpose, it was developed a calibration model based on an experimental design with 1 factor (meloxicam) and 5 levels (5 concentrations corresponding to 80%, 90%, 100%, 110% and 120% pharmaceutical active ingredient/TTS); in total 15 samples were prepared. For the validation set, 36 samples were prepared with concentrations corresponding to 90%, 100% and 110% meloxicam/TTS. Satisfactory results were acquired with the use of a spectral region 7872.5 - 5797.3 cm^{-1}, using first derivation (FD) followed by standard normal variate (SNV) pre-treatment method and 8 PLS (partial least squares) factors. According to the International Council for Harmonisation (ICH) guidance, the method was validated in terms of accuracy, precision and trueness. Compared to HPLC reference method, the value resulted by applying the NIR method showed no statistical differences ($p > 0.05$). Therefore, this method can be applied for the direct quantification of meloxicam from TTS. Also, as is a non-invasive and fast method, NIR spectroscopy is suitable to *on/at line* monitoring of the active substance content from TTS.

Rezumat

Această lucrare descrie dezvoltarea şi validarea unei metode NIR spectrometrice pentru dozarea directă a meloxicamului din sisteme terapeutice transdermice (STT). În acest scop, s-a dezvoltat un model de calibrare pe baza unui plan experimental cu 1 factor (meloxicam) şi 5 nivele (5 concentraţii corespunzătoare la 80%, 90%, 100%, 110% şi 120% substanţă activă/STT), fiind preparate astfel în total 15 probe. Pentru validare s-au preparat în total 36 de probe cu concentraţii corespunzătoare la 90%, 100% şi 110% meloxicam/STT. Rezultate satisfăcătoare s-au obţinut pe domeniul spectral 7872,5 - 5797,3 cm^{-1}, folosind ca şi metodă de pre-tratament prima derivată (FD), urmată de variaţia standard normal (SNV) şi 8 factori PLS (*partial least squares*). În conformitate cu prevederile ghidurilor ICH s-a validat metoda în termeni de acurateţe, precizie şi încredere. Comparativ cu o metodă HPLC de referinţă, valorile obţinute prin metodă NIR nu diferă statistic ($p > 0,05$). Prin urmare, această metodă NIR poate fi aplicată pentru dozarea directă a meloxicamului din STT, având avantajul că este neinvazivă pentru probă, rapidă şi poate permite monitorizarea *on/at line* a conţinutului de substanţă activă.

Keywords: meloxicam, NIR spectroscopy, PLS, transdermal therapeutic system

Introduction

The interest for incorporating active pharmaceutical ingredients into transdermal therapeutic systems (TTS) increased in the last years [1-4]. This modern pharmaceutical form is preferred to semi-solid or orally administrated pharmaceutical forms. TTS ensure predetermined doses and surface as compared to semi-solid forms. Compared to oral administration, they present multiple advantages, such as avoiding first pass and gastro-intestinal side effects, reducing the number of administrations or simply interrupting administration by removing the patch [5-8]. In the treatment of rheumatic diseases the first choice medication are non-steroid anti-inflammatory drugs (NSAID).

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazoyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is an enolic acid derivate belonging to the oxicam class, available on the pharmaceutical market as tablets, suppositories and injectable solutions. Meloxicam acts as a COX-2 selective inhibitor. Despite their efficiency in the rheumatic diseases, the risk of ulcerative side effects is relatively high. Accordingly to this, transdermal administration in TTS may be an alternative administration route. In the evaluation of TTS the determination of the active ingredient content is performed conventionally

by UV-VIS spectrometric methods or HPLC [9-14]. Both methods include a sample preparation step by dissolving the active pharmaceutical ingredient in different solvents, sonication, and preparing a large number of dilutions and filtration. All these procedures are time consuming. Near-infrared NIR spectroscopy is a novel method for active ingredient assay for various pharmaceutical forms, such as powder, granulates, tablets, gels and solutions. The advantages of NIR spectroscopy are short analysis time (less than a minute), low cost (no solvent consumption), and no sample preparation. Main disadvantages would be low signal sensibility (concentrations below 0.1% (w/w) are impossible to detect), high apparatus cost and in order to prove accuracy and robustness a high number of samples need to be prepared [15, 16]. In January 2014, European Medicines Agency (EMA) published a guide entitled: *"Guideline on the use of near infrared spectroscopy by the pharmaceutical industry and the data requirements for new submissions and variations"* [17]. According to this and various studies in the field, NIR spectra may be influenced by physical parameters such as: particle size, polymorphism, sample preparation, sample thickness demanding the application of mathematical pretreatments (normalization, derivatization) reducing the influence of these factors. Spectra pre-processing methods, defined as baseline correction-normalization [16], is used before development of calibration model, to decrease the side information contained in the spectra. The pre-processing methods applied in combination with the whole spectra or different spectral regions. For the development of prediction models is used partial least squares (PLS) regression. The number of PLS factors and the spectral pre-processing methods represents two important parameters in PLS algorithm [18-20]. Implementing a new method for meloxicam assay from TTS, such as NIR spectroscopy, represents an advantage for the pharmaceutical industry, because it is suitable to *on/at line* monitoring of the active ingredient content and because it offers the possibility of the directly determination of active pharmaceutical ingredient through a non-destructive mode of analysis. NIR spectroscopy is a method of analysis that, until now, has not been used to determine the drug content uniformity of TTS. This work describes the development and validation of NIR chemometric method for the quantification of meloxicam in polymeric films (TTS).

Materials and Methods

Materials. Meloxicam (MX) (Techno Drugs Intermediates, India) as active ingredient, hydroxypropyl methylcellulose 15000 (HPMC 15000, Shin-Etsu Chemical Co., Ltd, Japan) as polymer and 1,2-propylenglycol (PG) as plasticizer (Scharlau Chemie SA, Germany). Other materials: tween 20 (Sigma Aldrich, France), absolute ethanol (Chemical Company, Romania), and ultrapure water.

Sample preparation. Polymer films were prepared by solvent evaporation method. MX had been dissolved in a mixture of ethanol - PG (3:1 w/w) under continuous stirring (300 rpm). 1% tween 20 and 57% ultrapure water (w/w) was added to this solution, followed by the dispersion of polymer under 2 hours of stirring. The obtained mixture was then ultra-sonicated for 30 minutes and casted into 9.8 cm diameter Petri dishes. The films were dried at 40°C in the hot air oven for 24 h.

Calibration and validation protocol - MX quantification. For calibration 3 sets of film were prepared each of the having 5 concentrations of meloxicam: 80%, 90%, 100%, 110% and 120% corresponding to 0.40% w/w, 0.45% w/w, 0.50% w/w, 0.55% w/w and 0.60% w/w MX content. The three different batches were manufactured in three different days. The total number of films for calibration was 15. According to *Hubert et al.* [21, 22] for validation three batches of films were manufactured with 90%, 100%, 110% MX, each containing four films for every concentration level. In three different days, 36 films were prepared (Table I).

Table I

Calibration and validation design

Concentration level of meloxicam		Series 1		Series 2		Series 3	
		Calibration	Validation	Calibration	Validation	Calibration	Validation
0.40%	80%	1	0	1	0	1	0
0.45%	90%	1	4	1	4	1	4
0.50%	100%	1	4	1	4	1	4
0.55%	110%	1	4	1	4	1	4
0.60%	120%	1	0	1	0	1	0

Experimental conditions	Calibration				Validation			
	Series	Levels	Replicates	Total batches	Series	Levels	Replicates	Total batches
	3	5	1	15	3	3	4	36

NIR spectra recording. NIR spectra for the polymeric films were recorded using a MPA-NIR analyser (Bruker Optics, Germany) in Transmittance Sampling configuration. Each transmittance spectrum was

acquired by integrating 32 scans taken over a wave-number of 12500 to 5800 cm^{-1} with 16 cm^{-1} resolution.

NIR spectra processing. While mid-IR spectra and especially the absorbance bands are directly interpretable due to chemical peak specificity, NIR spectra are difficult to interpret. Therefore, the use of chemometrics is required [23]. For this purpose, different spectral pre-treatments in combination with different spectral ranges containing strong bands of MX were analysed [18-20, 24, 25]. The spectral pre-treatments tested in order to find the best calibration model included vector normalization: standard normal variate (SNV), straight line subtraction (SLS), minimum-maximum normalization (MMN), multiplicative scatter correction (MSC), first derivative (FD) and combinations of two pre-processing methods: FD followed by SNV, FD followed by MSC or FD followed by SLS. FD was obtained by applying Savitzky-Golay algorithm. Multivariate calibration was applied to chemometric approaches based on PLS (Partial Least Squares) regression using Opus Quant (Bruker Optics, Germany) software. The models were tested by full cross-validation. The calculation of the validation parameters: trueness, precision, accuracy, was performed using Microsoft Office Excel 2010 (Microsoft Corporation, USA).

Reference method. MX assay in TTS was performed using a HPLC-UV validated method. The chromatographic conditions were: column Waters C8 (4.6 x 150 mm, 5 µm); mobile phase phosphate buffer (KH_2PO_4 20 mM, pH 3.0):acetonitrile (60:40 v/v); 1.0 mL/min flow; detection at 362 nm; retention time was 3.08 min; column temperature was set at 35°C.

Results and Discussion

MX assay. The main aim of this study was to develop and validate a NIR-chemometric method suitable for the direct quantification of MX in polymeric films. This method compared to conventionally used determination methods is rapid, economic, non-toxic (does not involve organic solvents) and the samples are recovered.

Table II

Type of spectral pre-treatment, spectral range selected, number of PLS factors, RMSECV and Bias of different models for MX assay in polymeric films

Model	Pre-treatment	Spectral range selected (cm^{-1})	Number of PLS factors	R^2	RMSECV	Bias
a	None		10	84.60	0.035	0.0018
b	SNV	**7872.5 - 5797.3**	10	94.28	0.027	-0.0016
c	**FD+SNV**		**8**	**98.38**	**0.023**	**-0.0028**
d	None		10	85.78	0.034	-0.0500
e	FD	8127 - 5797.3	10	94.89	0.026	0.0015
f	FD+MSC		9	97.67	0.023	-0.0050
g	None		10	80.11	0.038	-0.0014
h	SNV	8967.9 - 5797.3	10	90.44	0.030	0.0086
i	FD+SNV		10	97.88	0.033	-0.0032
j	None		9	73.05	0.042	0.0014
k	MSC	12173 - 5797.3	8	84.51	0.034	-0.0019
l	FD+MSC		10	98.03	0.023	0.0019

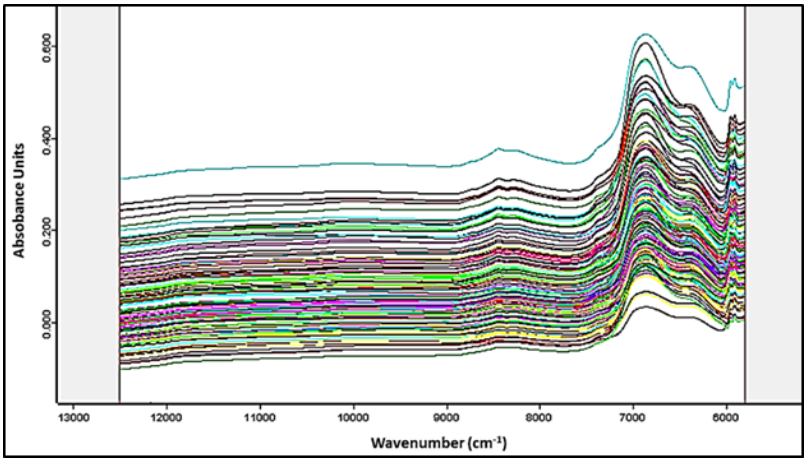

Figure 1.
NIR transmittance spectra of calibration samples

Spectra investigation. As seen in Figure 1, the intensive spectral peaks of MX are mainly in the region 12173 - 5797 cm^{-1}. Before applying any pre-treatment methods, four spectral regions (7872.5 -

5797.3 cm^{-1}, 8127 - 5797.3 cm^{-1}, 8967.9 - 5797.3 cm^{-1}, 12173 - 5797.3 cm^{-1}) were selected for the development of a model for MX assay.

Model selection and development. Model development for MX assay involved association of several spectral pre-treatment methods (None, SNV, FD, MSC, FD+SNV, FD+MSC) with different spectral regions. 12 potential models (Table II) were generated to develop a quantification model of MX in polymeric films. A widespread multivariate calibration technique (PLS regression) was performed to the calibration set and cross validation procedure was applied to choose the model with the biggest predictive potential. According to the specifications of Haaland and Thomas, the selection of an optimum number of PLS factors is very important to avoid *over fitting* [26]. The best fitting model was selected by calculating RMSECV (root mean square error of cross validation), bias (experimental error) and R^2 (high determination coefficient). The validation model selection considered the smallest value of RMSECV, bias values approaching zero, R^2 values approaching 1 and low number of PLS [27-30].

$\mathbf{RMSECV}=\sqrt{\frac{PRESS}{n}}$	$\mathbf{BIAS}=\frac{1}{N}\sum(Xn-Yn)$
$PRESS=\sum(Ypred-Ytrue)^2$	Xn – actual values in the calibration set
PRESS - predicted residual error sum square	Yn – predicted values in the calibration set
Ytrue - true concentration of the drug	
Ypred - predicted concentration of the drug	
n - number of training samples	

Taking into consideration the most relevant statistical parameters: highest R^2 and lowest RMSECV, the model c (FD+SNV) based on spectral region 7872.5 - 5797.3 cm^{-1} showed the best ability of prediction using 8 PLS factors, and was selected for further method validation.

Validation of the method. For calibration, samples were prepared at three different active content level 0.45% w/w, 0.50% w/w and 0.55% w/w corresponding to 90%, 100%, 110% MX, in four independent batches, resulting in a total of 36 spectra. Figure 2 presents the accuracy profile for the quantification of MX in polymeric films, which relies on the results of validation for model c (FD+SNV), first derivative followed by standard normal variate pre-processing, in spectral region 7872.5 - 5797.3 cm^{-1}. According to ICH Q8 (R2) guideline requirements [31], validation protocol implies evaluating the trueness, precision and accuracy. Trueness was evaluated by calculating relative bias and the recovery. Validation results, presented in Table III, show a recovery of almost 100% with variability between 96.66% and 101.71%.

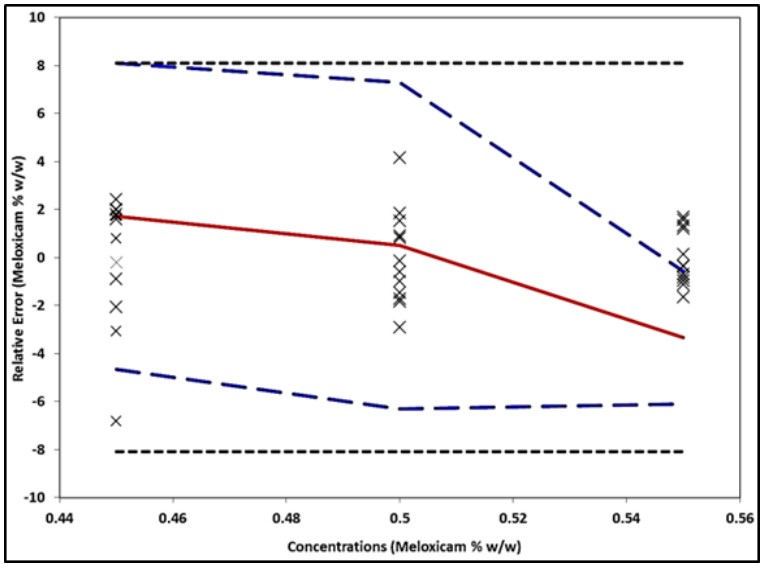

Figure 2.

Accuracy profile for MX assay, based on validation results obtained with model c: the plain line is the relative bias; the dashed lines are the β-expectation tolerance limits (β = 95%); the dotted curves are the acceptance limits (± 8.1%)

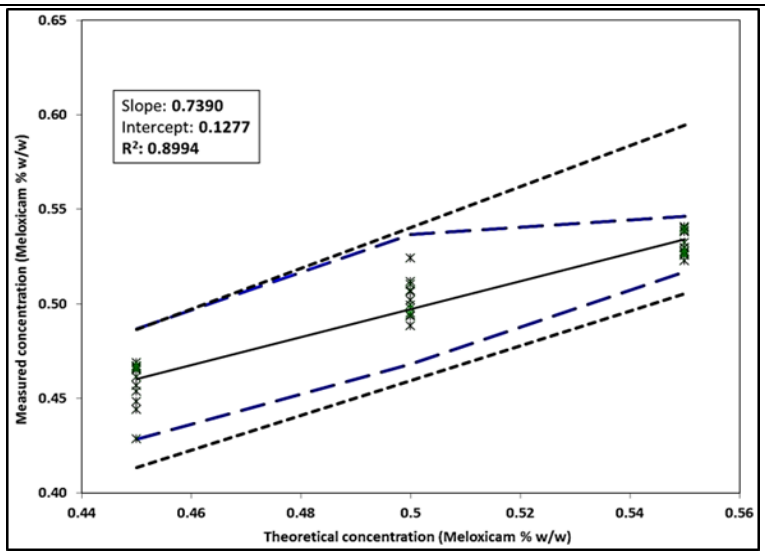

Figure 3.

Linear profile of NIR model MX assay: the dashed limits on the graph correspond to the accuracy profile and the dotted curves represent the acceptance limits (± 8.1%); the continuous line is the identity line y = x

Table III

Validation results of the NIR method for the quantification of MX in polymeric films

	Trueness		Precision		Accuracy	
Concentration level (% MX)	**Relative bias (%)**	**Recovery (%)**	**Repeatability (RSD %)**	**Intermediate precision (RSD %)**	**Relative tolerance limits (%)**	**Tolerance limits (% in polymeric films)**
0.45	1.712	101.71	2.56	2.72	[-4.66, 8.08]	[0.42, 0.48]
0.50	0.493	100.49	1.32	2.19	[-6.30, 7.29]	[0.46, 0.53]
0.55	-3.343	96.66	1.13	1.18	[-6.11, -0.57]	[0.51, 0.54]

The precision of the method was analysed by calculating repeatability (intra-day precision) and intermediate precision (repeatability over different days). Both parameter values are satisfying the criteria for all levels of active ingredient content. An inverse proportioned dependence to the MX concentration is observed. The best repeatability value was obtained at 0.55% w/w MX. As shown in Figure 2 and Table III, accuracy has the largest tolerance limit at [-6.30%, 8.09%] and the best relative tolerance [-6.30%, 7.29%] for 0.5% w/w MX. The linearity of the method is confirmed by the high R^2 value (89.94) and the slope close to 1 (0.7390).

The results reveal that the NIR chemometric method applied by using model c (FD+SNV) is sufficiently precise and accurate for the assay of MX in polymeric films.

Similar results were obtained in other studies in which there were implemented NIR methods for quantification of MX on intact tablets or on powder blends for tableting [13, 15]. Based on the statistical parameters acquired in the validation process, it can be concluded that this method can be applied for the assay of MX from TTS.

Application of the method. After validation, the NIR method were applied on four control samples containing 0.50% w/w MX/TTS and the results were compared with the values obtained by the HPLC reference method (Table IV).

Table IV

MX content of control samples by NIR method and HPLC reference method

MX in control samples (% w/w /TTS)		**NIR method**	**HPLC method**
Determined		0.495	0.480
		0.493	0.469
		0.494	0.483
		0.488	0.489
	Mean ± SD	0.493 ± 0.0031	0.480 ± 0.0083
	***t-value**	2.598	
	***p-value**	0.080	
		(statistically insignificant for 95 % CI)	
Recovered	**Mean ± SD**	98.54 ± 0.62	96.06 ± 1.67

SD = Standard Deviation; CI = Confidence Interval; * Student's t test

The results showed an active content recovery of 98.54% by NIR method compared to 96.06% by HPLC method. Student's t test demonstrated no statistical difference ($p > 0.05$) between the two methods.

A frequent approach in developing a NIR chemometric method for quantification of an active pharmaceutical ingredient consists in the validation of this method compared to a HPLC method as reference by proving the similarity of results. For example, it has been shown no statistical differences between these two methods, comparing the results acquired for the quantification of atorvastatin and amlodipine in powder blends for tableting [25]. The same conclusion was drawn in another study that aimed to develop and validate a NIR method for the direct quantification of meloxicam in intact tablets [13]. These studies certify that a conventional method used for assay of an active pharmaceutical ingredient can be successfully replaced by NIR method.

Conclusions

In this study a NIR method was developed for the quantification of MX incorporated in HPMC polymeric matrix for transdermal delivery. In the developing process, different pre-treatment methods were tested and the method was fully validated according to the ICH Q8 (R2) guidance [31] for model c (FD+SNV). The results demonstrated good linearity, accuracy, precision and trueness for the determination of MX from polymeric matrix presented in form of films with 90 - 110% active pharmaceutical ingredient content. Concerning the accuracy of the method the limits were below ± 10%, thus this method can be applied for the quantification of MX without sample preparation and used *on/at line* monitorization of the technological process of polymeric film manufacturing. The results obtained in this work showed that the proposed method has a good similarity with HPLC reference method used for MX assay. In conclusion, the new developed NIR spectroscopy method is suitable for rapid quantification of MX in TTS without any sample preparation.

References

1. Rostogi V., Yadav P., Transdermal drug delivery system: An overview. *Asian J. Pharm.*, 2012; 6: 161-170.
2. Kranthi Kumar K., Sasikanth K., Sabareesh M., Dorababu N., Formulation and evaluation of diacerein cream. *Asian J. Pharm. Clin. Res.*, 2011; 4(2): 93-98.
3. Ruela A.L.M., Figueiredo E.C., Perissinato A.G., *In vitro* evaluation of transdermal nicotine delivery systems commercially available in Brazil. *B.J.P.S.*, 2013; 49(3): 579-588.
4. Singh I., Morris A.P., Performance of transdermal therapeutic systems: Effects of biological factors. *Int. J. Pharm. Investig.*, 2011; 1(1): 4-9.
5. Cilurzo F., Gennari C., Minghetti P., Adhesive properties: a critical issue in transdermal patch development. *Expert. Opin. Drug Deliv.*, 2012; 9(1): 33-45.
6. Inayat B.P., Setty C.M., Chemical penetration enhancers for transdermal drug delivery systems. *Trop. J. Pharm. Res.*, 2009; 8(2): 173-179.
7. Gaikwad A.K., Transdermal drug delivery system: Formulation aspects and evaluation. *J. Pharm. Sci.*, 2013; 1(1): 1-10.
8. Popovici I., Lupuleasa D., Pharmaceutical Tehnology, vol. 3. Ed. Polirom, București, 2009; 669-674, (available in Romanian).
9. Cristea A.N., Pharmacological compendium. Ed. Medicală, Bucuresti, 2009; 617-633, (available in Romanian).
10. Shirsand S.B., Ladhane G.M., Prathap S., Prakash P.V., Design and evaluation of matrix transdermal patches of meloxicam. *RGUHS J. Pharm. Sci.*, 2012; 2(4): 58-65.
11. Duan X.D., Ji C.J., Nie L., Formulation and development of dendrimer-based transdermal patches of meloxicam for the management of arthritis. *Trop. J. Pharm. Res.*, 2015; 14(4): 583-590.
12. Somasundaram J., Sundarapandian R., Posina A., Design and evaluation of monolithic drug-in-adhesive transdermal patches of meloxicam. *Malay. J. Pharm. Sci.*, 2010; 8(2): 25-43.
13. Tomuţă I., Iovanov R., Bodoki E., Leucuţa S.E., Quantification of meloxicam and excipients on intact tablets by near infrared spectrometry and chemometry. *Farmacia*, 2010; 58(5): 559-571.
14. Csifo (Vajda) E., Nagy E.E., Horváth E., Fárr A., Muntean D.L., Mid-term effects of meloxicam on collagen type II degradation in a rat osteoarthritis model induced by iodoacetate. *Farmacia*, 2015; 63(4): 556-560.
15. Tomuţă I., Iovanov R., Vonica A.L., Leucuţa S.E., High-throughput NIR-chemometric method for meloxicam assay from powder blends for tableting. *Sci. Pharm.*, 2011; 79: 885-898.
16. Jamrógiewicz M., Application of the near-infrared spectroscopy in the pharmaceutical technology. *J. Pharm. Biomed. Anal.*, 2012, 66: 1-10.
17. ***The European Agency Science Medicines Health, 27 January 2014; EMEA/CHMP/CVMP/QWP/17760/2009 Rev2.
18. Porfire A., Muntean D., Achim M., Vlase L., Tomuţă I., Simultaneous quantification of simvastatin and excipients in liposomes using near infrared spectroscopy and chemometry. *J. Pharm. Biomed. Anal.*, 2015; 107: 40-49.
19. Porfire A., Rus L., Vonica A.L., Tomuţă I., High-throughput NIR-chemometric methods for determination of drug content and pharmaceutical properties of indapamide powder blends for tabletting. *J. Pharm. Biomed. Anal.*, 2012; 70: 301-309.
20. Porfire A., Tomuţă I., Tefas L., Leucuţa S.E., Achim M., Simultaneous quantification of L-α-phosphatidylcoline and cholesterol in liposomes

using near infrared spectrometry and chemometry. *J. Pharm. Biomed. Anal.*, 2012; 63: 87-94.
21. Hubert Ph., Hguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal-part II. *J. Pharm. Biomed. Anal.*, 2007; 45 (1): 70-81.
22. Hubert Ph., Hguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal-part III. *J. Pharm. Biomed. Anal.*, 2007; 45(1): 82-96.
23. Roggo Y., Chalus P., Maurer L., Lema-Martinez C., Edmond A., A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. *J. Pharm. Biomed. Anal.*, 2007; 44: 683-700.
24. Tomuţă I., Rus L., Iovanov R., Rus L.L., High-throughput NIR-chemometric methods for determination of drug content and pharmaceutical properties of indapamide tablets. *J. Pharm. Biomed. Anal.*, 2013; 84: 285-292.
25. Vonica-Gligor A.L., Casian T., Reznek A., Tomuţă I., Gligor F., Simultaneous quantification of atorvastatin and amlodipine in powder blends for tableting by NIR spectroscopy and chemometry. *Farmacia*, 2015; 63(3): 381-387.
26. Haaland D.M., Thomas E.V., Partial Least-Squares Methods for Spectral Analyses. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information. *Anal. Chem.*, 1988; 60: 1193-1202.
27. Mantanus J., Ziémons E., Lebrun P., Active content determination of non-coated pharmaceutical pellets by near infrared spectroscopy: method development, validation and reliability evaluation. *Talanta*, 2010; 80: 1750-1757.
28. Burns D.A., Cinczak E.W., Handbook of New-Infrared Analysis. Ed CRC Press Taylor & Francis Group, 3rd ed., New York, 2008; 220-221.
29. Mukherjee Pulok K., Evidence-based validation of herbal medicine. Ed. Elsevier, India, 2015; 483.
30. Ferreira D.S., Pallone J.A.L., Poppi R.J., Fourier transform near-infrared spectroscopy (FT-NIRS) application to estimate *Brazilian soybean* [Glycine max (L.) Merril] composition. *Food Research International*, 2013; 51: 53-58.
31. ***ICH Harmonized Tripartite guideline: Pharmaceutical Development, Q8 (R2), http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf.