ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2017, *62*(4-5), 365-372

Dedicated to Professor Ionel Haiduc
on the occasion of his 80th anniversary

SIMULTANEOUS QUANTIFICATION OF SOME NON-STEROIDAL ANTI-INFLAMMATORY AND ANALGESIC DRUGS BY PRINCIPAL COMPONENT REGRESSION

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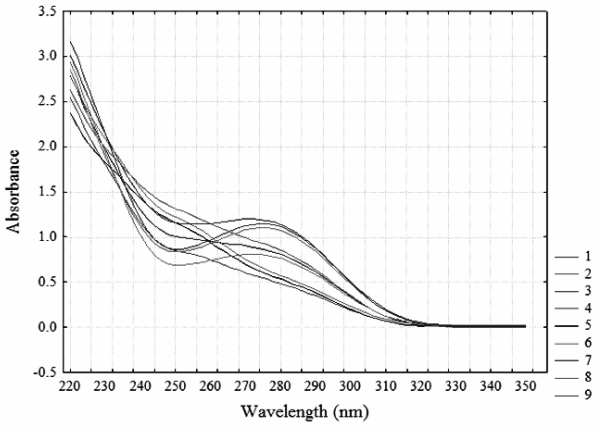
Received December 20, 2016

During this study the simultaneous determination of paracetamol, sodium diclofenac and ibuprofen by a multivariate method that combines the Plackett–Burman calibration design with the principal component regression is presented. The quality of models and their predictive capacity have been investigated by various statistical indices. The reliability of models has been proved by analyzing real samples using two procedures. The first procedure involved the direct investigation of samples, while the second one involved the addition of a known amount of standards in the real samples. The very good results obtained for both synthetic and real samples have demonstrated that this multiple regression method may be successfully extended for quality control in pharmaceutical products and also for routine investigations. Furthermore, the presented protocol may be easily extended in the analysis of different compounds from complex samples.



INTRODUCTION

Nowadays, the increasing rate of pharmaceutically active compounds release in the environment, which has a significant influence on the human and animal health, has lead to real analytical challenges.[1-5] Some of the most controversial classes of pharmaceuticals that present high risk of environment accumulation are the antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics and endocrine disrupting compounds.[6-9] The NSAIDs are usually used for their anti-inflammatory action, and also as antipyretic agents. Still, they are known for causing some serious side effects, such as gastrointestinal injuries and they also could provoke some cardiovascular complications.[9] However, they are worldwide commercialized in very high amounts, i.e. in Germany in 1998 the total quantity of aspirin sold was about 500 tones, 75 for diclofenac and 180 for ibuprofen.[10] The considerable consumption, high water solubility and the incomplete removal during conventional treatments of sewage waters have induced the increased water environments contamination with concentration levels of low

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ng L^{-1} up to μg L^{-1}.[11] During the wastewater treatment procedures the majority of the pharmaceutical compounds are not completely mineralizing and, therefore, they pass through the wastewater-treatment plant and end up in the receiving waters. Their removal is variable and usually depends on the compounds properties and elimination process conditions (sludge retention time, hydraulic retention time, temperature, and so on). This delicate problem has been already considered by the scientific world and there has been offered some solutions applicable at laboratory scale. However, up today they were not extended to industrial level.[12] Taking into consideration these facts, it is easy to understand that the necessity of competitive quantification methods is relevant. The complexity of environmental and biological samples requires often laborious sample preparation protocol that may lead to decreased accuracy of the methods, and implicitly to inadequate characterization of the analyzed samples. Moreover, even if the sample preparation does not induce significant errors, the determination methods are complex and involve frequently very expensive detection techniques (MS-MS, NMR etc.). Such results are usually highly significant and generally accepted as being relevant, but the costs of analysis are very high.[13-15] Therefore, the interest in the development of methods for simultaneous analysis, which do not involve a preliminary separation step, suitable for the routine pharmaceutical analysis, is justified. In this context, the solution is given by development of chemometric methods, which include the experimental design and exploratory data analysis.

The chemometric approaches are able to extract and distinguish the specific compound response from raw information. Any quantitative determination method involves a calibration step, which may be univariate or multivariate. The most popular and powerful chemometric methods of determination, like principal component regression (PCR) or partial least square (PLS) are recently using the multivariate calibration, consisting of the minimal representative combination that describes correctly a given system. The most important aspects of the experimental design development are largely presented in the literature.[16] The chemometric methods like PCR and PLS, are highly compatible with spectroscopic methods (UV-Vis, IR, Raman, etc.), that usually furnished very discreet compounds discrimination data from a complex matrix. The highest advantage of these methods is the possibility of simultaneous determination of structurally related compounds, without a previous separation step, and in addition the sample preparation protocol may be considerably simplified.[17-20] In the particular case of NSAIDs and analgesics, there are only a few PCR studies.[21]

The multivariate regression methods consider that a series of variables describes a system. For example, in the PCR procedure the concentration is described by an undetermined number of scores corresponding to the first principal components (PCs), obtained by applying the algorithms of principal component analysis (PCA) over the digitized chemical information, i.e. spectra or chromatograms.[22-27]

The aim of the present work is focused on the development of a method for simultaneous quantification of paracetamol, ibuprofen and sodium diclofenac by multivariate calibration. The developed method will be based on an experimentally design approach combined with PCR methodologies. The training set was carefully chosen using a Plackett–Burman calibration design at three levels of concentration. The precision and the accuracy of the developed method would be evaluated through several statistical parameters. The developed method presents the serious advantage of allowing the simultaneous determination of compounds, without involving a separation or a complex sample preparation step prior to analysis. This method may be further elaborated to be useful in environmental and biological analysis, or for quality control in pharmaceutical products.

RESULTS AND DISCUSSION

Prior to the quantitative analysis some important parameters were selected. The first one was the UV domain where all the analytes may be observed. The most specific region was between 220 to 350 nm. The standards concentration level was chosen according to the UV system limitation, so the spectrum corresponding to the solution with all the drugs in highest concentration had to be less than 4, while in case of the mixture corresponding of the lowest amounts the specific characteristics of the spectrum must be observed. The zero order spectra of absorption corresponding to paracetamol (11 μg mL^{-1}), sodium diclofenac (22 μg mL^{-1}), ibuprofen (32 μg mL^{-1}) and their mixture are

presented in Fig. 1. Each compound presents characteristic UV spectrum, with maximum absorption at 245 nm for paracetamol, 276 nm for sodium diclofenac and 224 nm for ibuprofen. However, the spectra are relatively simple and they are not characterized by very specific particularities. In case of ibuprofen the spectrum presents very low information since between 220 and 225 nm a large variety of chemical entities are leading to absorption. The spectrum corresponding to their mixture is very simple and indicates that a direct evaluation of these compounds will be impossible without using an advanced chemometric approach.

The careful investigation of the spectra points out that the solution may be offered by multivariate analysis and experimental design. According to Brereton,[16] nine solutions (Table 1 and Fig. 2) are enough to describe a system with three components when the multivariate analysis involved is PCR.

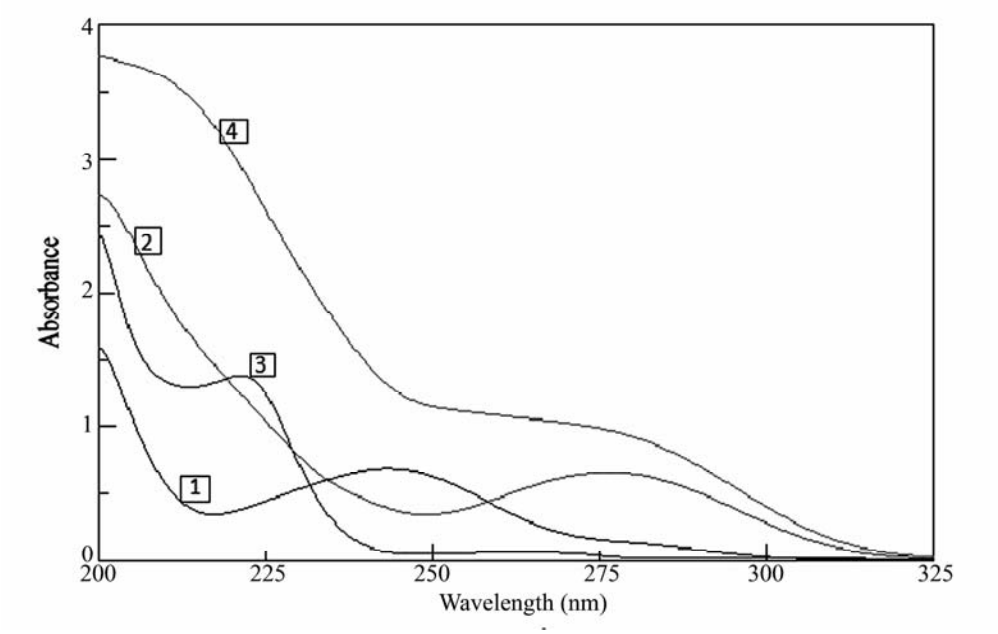

Fig. 1 – The UV absorption spectra of paracetamol (1), sodium diclofenac (2), ibuprofen (3) and their mixture (4).

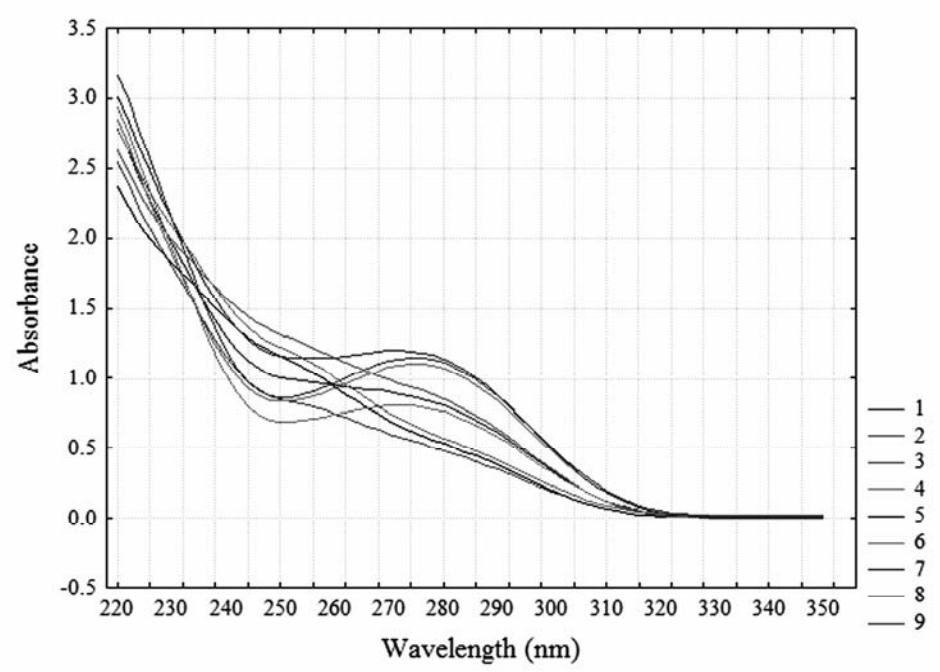

Fig. 2 – The Plackett–Burman calibration profile (1-9 numbers corresponding to the calibration solution).

Table 1

The training and test sets for the three-component system

No	Sample	Paracetamol (μg mL^{-1})	Diclofenac (μg mL^{-1})	Ibuprofen (μg mL^{-1})
1		10	20	30
2		15	20	20
3		10	10	40
4	Multivariate	5	30	20
5	calibration	15	10	30
6		5	20	40
7		10	30	20
8		15	10	40
9		5	30	30
10	Synthetic	11	22	32
11	Samples	11	22	32
12		11	22	32

Prior to the multiple regression, the PCA has been applied on the matrix formed by the digitized spectra of the solutions, including the test set or real samples spectra. Based on quality statistical coefficients have been selected the optimal number of factors retained so that the regression function between the scores of the principal components and the concentration would have the highest ability of prediction without over fitting the concentration data. The quality of the prediction model obtained by applying PCR on the obtained principal components can be judged by the performance criteria. The accepted indices may be classified in two groups: the first one being formed by the classical indices that describe how well they fit the linear models (correlation coefficient: r, determination coefficient: R^2, F value, standard deviation: SD, and so on), but they are not that relevant in case of multivariate regression. However, the results may also be sustained by the second group formed by the relevant indices of quality that describe to which grade they fit and their prediction power, such as cross validated determination coefficient (Q^2), standard deviation error in calculation (SDEC), standard deviation error of prediction (SDEP), the prediction sum of squares (PRESS) or root sum square (RSS). The selected statistical indices for our particular case are presented in Table 2. The very good values of these parameters are indicating that the obtained models may be used for the evaluation of real samples. The obtained indices are sustaining the high quality of the models.

The obtained models were evaluated considering their applicability on test and real samples. The method has been validated by investigating its precision and accuracy. The precision has been investigated by means of repeatability observed in case of three parallel synthetic samples (test set). The mean values of the obtained concentrations near standard deviation values (SD) are listed in Table 3.

The obtained values are suggesting that the developed method is characterized by a high level precision. Moreover, the accuracy of the analytical procedure has been evaluated through the mean recovery values, which were of 101.00% (± 0.05) for paracetamol, 100.19% (± 0.05) for diclofenac and 101.28% (± 0.14) for ibuprofen. All the exposed values are indicating that the method is highly accurate and precise.

Table 2

The statistical parameters concerning the obtained PCR models quality and their predictive capacity

Statistical parameter	Statistica			Moby Digs		
	Paracetamol	Sodium Diclofenac	Ibuprofen	Paracetamol	Sodium Diclofenac	Ibuprofen
No. of PCs*	6	5	6	3	3	3
Q^2	0.9999	0.9999	0.9999	0.9969	0.9980	0.9966
R^2	0.9999	0.9999	0.9999	0.9991	0.9995	0.9990
SD	0.008	0.023	0.016	0.164	0.251	0.344
F	415174	219619	405187	1865	3160	1690
SDEP	0.0314	0.0898	0.0261	0.2282	0.3694	0.4736
SDEC	0.037	0.0135	0.0074	0.1220	0.1875	0.2563
PRESS	0.009	0.073	0.006	0.468	1.228	2.019
RSS	0.000	0.002	0.000	0.134	0.316	0.591

* No of PCs – number of PCs accepted in the final models of prediction.

Table 3

Results concerning the precision and the accuracy of the method (N = 3)

Added (μg mL^{-1})			Found (μg mL^{-1})		
Paracetamol	Sodium diclofenac	Ibuprofen	Paracetamol	Sodium diclofenac	Ibuprofen
11.00	22.00	32.00	11.16	22.03	32.26
11.00	22.00	32.00	11.08	22.10	32.55
11.00	22.00	32.00	11.09	22.00	32.43
Mean concentration (μg mL^{-1})			11.11	22.04	32.41
SD			0.04	0.05	0.15
Mean recovery (%)			101.00	100.19	100.28
SD			0.05	0.05	0.14

Table 4

The pharmaceutical products information

No	Product Name	Active Compound	Amount of active compound/ tablet (mg)	Other Components	Producer
1	Paracetamol LPH®	Paracetamol	500	Maize starch, Povidone K30, Silicium dioxide anhydrous colloidal, Magnesium stearate, Talc	LaborMed Pharma (Romania)
2	Diclac®	Diclofenac sodium	75	Lactose, Methyl hydroxypropyl cellulose, Microcrystalline cellulose, Calcium hydrogen phosphate dehydrate, Starch, Sodium starch glycolate**,** Silicium dioxide anhydrous colloidal, Magnesium stearate, Iron oxide red, Purified water	Salutas Pharma GmbH (Germany)
3	Ibuprofen	Ibuprofen	200	Starch, Lactose, Methylcellulose, ***Sodium starch glycolate,*** Magnesium stearate, Silicium dioxide anhydrous colloidal, Hypromelose film, Talc, Titanium dioxide, Propylene glycol, Synthetic die (Erytrosine)	Cipla Limited (India)

One may claim that the method has been tested on synthetic samples, which are not representing the matrix complexity of real samples. In this context the method has been advanced for real samples of pharmaceutical products (Table 4). For each pharmaceutical were prepared four parallel samples. The solutions were prepared in such manner that the concentration was in the middle of the selected concentration domain. The obtained results are depicted in Table 5.

There can be observed that the method is able to differentiate the UV answer resided from the tablets matrix upon that corresponding to the investigated compounds. However, the highest recovery (> 93.50 %) calculated upon of the labeled amount from a tablet, could be observed for ibuprofen, followed by paracetamol and finally sodium diclofenac. Often the UV determination of ibuprofen is classified as being very difficult, especially when the involved techniques are the chromatography or simple UV-Vis spectroscopy. This is a consequence of ibuprofen's low absorbance and also because of it's the absorbance domain. In this particular case, by combining the UV-Vis spectroscopy and the multivariate chemometric approaches, some substantial improvements may be observed.

The proposed method, overcame this difficulties for ibuprofen determination, even if the discrimination is made against the paracetamol, which is a pharmaceutical compound that is usually combined with ibuprofen in various formulations. The method is also very efficient in the paracetamol evaluation, where the recovery has been higher than 93.98%. In the case of sodium diclofenac, there may be observed that the found level is always higher than the claimed label. Since the found level in synthetic samples was very close of the theoretical one, this increased value may be considered a consequence of the matrix effect.

Table 5

The method evaluation by means of real samples

Compound	Sample No	Weighed tablet (g)	Claimed label (mg)	Found (mg)	Found (spiked samples) (mg)	Recovery (%)	Recovery (spiked samples) (%)
Paracetamol	1	0.607	500	478.25	467.50	95.65	93.50
	2	0.608	500	489.20	455.00	97.84	91.00
	3	0.598	500	469.90	462.50	93.98	92.50
	4	0.606	500	477.65	468.33	95.53	93.67
Mean value of found amount and recovery				478.75 (± 7.94)	463.33 (± 6.12)	95.75	92.67
Sodium diclofenac	1	0.222	75	82.50	76.44	110.00	101.92
	2	0.211	75	81.75	77.54	109.00	103.39
	3	0.207	75	79.81	83.48	106.40	111.31
	4	0.209	75	80.44	84.40	107.25	112.54
Mean value of found amount and recovery				81.13 (± 1.22)	80.47(± 4.05)	108.16	107.29
Ibuprofen	1	0.325	200	193.88	188.20	96.94	94.10
	2	0.327	200	197.63	193.00	98.81	96.50
	3	0.317	200	189.81	187.40	94.91	93.70
	4	0.316	200	187.00	188.40	93.50	94.20
Mean value of found amount and recovery				192.08 (± 4.65)	189.25 (± 2.54)	96.04	94.63

In addition, to confirm the obtained results, a parallel procedure has been developed. A well known amount of compounds has been added to the real samples. These procedures were meant to evaluate once more if the found amount is corresponding to that existed in the tables. The final result was obtained by decreasing the added amount from the measured content. The obtained results are presented also in Table 5. However, the obtained results are highly similar, but in all cases a bit lower recovery could be observed. This may be a consequence of the extra steps involved in the sample preparation procedure.

EXPERIMENTAL

Materials and Reagents

The paracetamol standard was obtained as a donation from LaborMed Pharma (Romania), the ibuprofen was purchased from Basf Aktiengesellscharf (Germany), while the diclofenac sodium has been obtained from Henan Dongtai Pharm Co (China). The structure of the investigated compounds is presented in Fig. 3.

The involved solvents were methanol (Chemical Company, Roumania) and distilled water. All chemicals and solvents were of analytical grade.

Equipments and Software

The UV spectra were registered with a UV-VIS Jasco double beam spectrophotometer, V-50 model, (Easton, MD, USA) equipped with a deuterium lamp for the UV and a halogen lamp for visible domain. The slit was fixed at 0.5 nm. The 10 mm path length cells were used to obtain the spectra of all the solutions. Other characteristics of the system are: the registering speed (400 nm min^{-1}), wavelength precision (± 0.3 nm), photometric accuracy (± 0.004), and the wavelength reproducibility (± 0.1 nm). The spectra were registered in the UV domain, from 220 nm to 350 nm. The spectrophotometer was connected to a computer loaded with the Spectra Manager software, which was used to process the spectral data. The multidimensional analyses that involved various statistical approaches were performed by using the Statistica 7 (www.statsoft.com) and MobyDigs (www.talete.it) software.

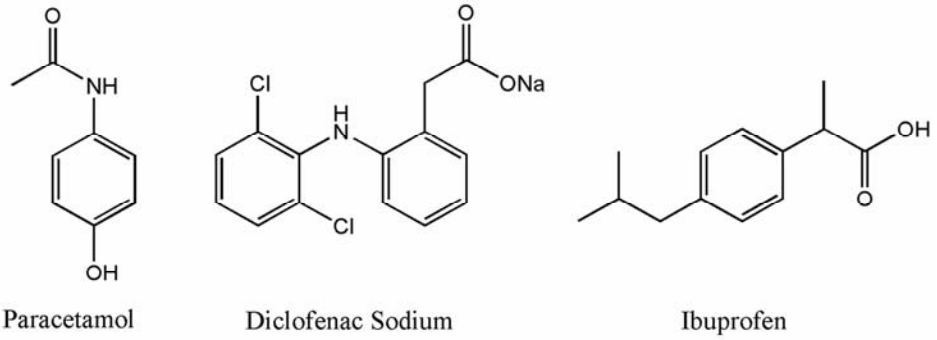

Fig. 3 – The chemical structure of the studied compounds.

Experimental Design

The stock solutions (1 mg mL^{-1}) of paracetamol, sodium diclofenac and ibuprofen were daily prepared in methanol. The corresponding amount of standards was carefully weighted in volumetric flasks of 100 mL, over which was added 50 mL of methanol. In order to obtain a homogeneous solution and to be sure that the entire amount of standard is dissolved, the solution was sonicated for 20 minutes in an Elmasonic S15H ultrasonic bath, with a frequency of 37 kHz and a power of 95 W. After the sonication step, the volumetric flasks were filled up to quote with methanol. After that, from the solutions resulted were prepared the stock solutions in a concentration of 100 μg mL^{-1} in 500 mL volumetric flask that were filled up with distilled water. The calibration solutions were prepared in volumetric flasks of 100 mL by mixing the adequate volumes from the stock solution of each compound. The training set from the multivariate calibration has been selected according to a Plackett–Burman design method described by Brereton[16] and the concentrations involved were of three levels. The training set was consisting of 12 samples (Table 1). The nine training set solutions were chosen by using the calibration design method previously mentioned. The concentration level of standards in the calibration set was as follows: 5-15 μg mL^{-1} for paracetamol, 10 - 30 μg mL^{-1} for sodium diclofenac and finally 20 - 40 μg mL^{-1} for ibuprofen.

Sample Preparation

The developed method has been validated by synthetic samples (test set from Table 3), real samples and real samples with standard addition. The concentration of the synthetic samples was chosen near by the middle of the selected concentration domain. The three synthetic samples were prepared directly from the stock solution in volumetric flasks of 100 mL and they were used to estimate the recovery and also the accuracy. In addition, the method has been tested on three pharmaceutical products, available on the Romanian market. The information regarding the investigated drugs is listed in Table 5.

Four tablets of each drug were weighed and then powdered by means of a mortar and pestle. The homogeneous powder was weighed in 250 mL volumetric flask and then dissolved with methanol under ultrasonic conditions for 25 minutes. The obtained solutions were filtered through paper filter in order to eliminate the insoluble ingredients. From the filtrate there has been prepared solutions with a concentration of 100 μg mL^{-1}. These solutions were used for the preparation through dilution of the samples that were analyzed by spectroscopy method. For each pharmaceutical product, four parallel samples were investigated. The UV spectra were registered upon of a blank solution consisting in distilled water. The 100 μg mL^{-1} solutions of pharmaceuticals were used also for validation by standard addition method.

CONCLUSIONS

The proposed method has proved to be very efficient in the simultaneous analysis of paracetamol, sodium diclofenac and ibuprofen. According to the obtained statistical parameters this method, obtained by combining the UV spectroscopy with Plackett–Burman calibration design and multivariate analysis, is highly precise and accurate. Moreover, the high quality statistical indices R^2, Q^2, SD, F, SDEC, SDEP, SDEC, PRESS and RSS have justified the quality of regression models and their predictive capacity. The recoveries obtained are indicating that the method is a very useful tool in the analysis of the investigated pharmaceuticals, especially for ibuprofen. The very good results obtained for both synthetic and real samples have demonstrated that this multiple regression method may be successfully extended for quality control in pharmaceutical products and also for routine investigations. Moreover, the method is sustained by the fact that it requires unsophisticated equipments, which can be easily operated without special training. In the future the presented protocol may be refined in order to be applied to more complex samples, such as the environmental or biological once.

Acknowledgments: This work was possible with the financial support of the Sectorial Operational Programme for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU 89/1.5/S/60189 with the title 'Postdoctoral Programs for Sustainable Development in a Knowledge Based Society' and the financial support offered by Roumanian Ministry of Education, Research, Youth and Sport through research grant PN-II-ID-PCE-2011-3-0366 (Project Manager: C. Sârbu).

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