UNCERTAINTY ESTIMATION FOR ACETAMINOPHEN SPECTROPHOTOMETRIC DETERMINATION AFTER THE TRANSPORT THROUGH BULK LIQUID MEMBRANE

Ioana DIACONU[1], Cristina Monica MIREA[2], Elena RUSE[3], Andreea Maria BALEA[4]

The measurement uncertainty is a very important parameter in establishing the quality of a measurement. Uncertainty plays a decisive role in conformity-nonconformity studies, commercial or legal action. In this study a bottom-up approach was realized to estimate the uncertainty. A cause-effect diagram is used to determine the uncertainty sources. Also a good description of the process helps to estimate the uncertainty. The uncertainty budget is established defining the parameter with a major contribution to the sample uncertainty. The final result will be presented as follows: $2.8829 \cdot 10^{-4} \pm 3.2833 \cdot 10^{-7}$ g.

Keywords: uncertainty, cause-effect diagram, acetaminophen, spectrophotometric determination, bulk liquid membrane

1. Introduction

Acetaminophen was first introduced in medicine by Von Mering in 1893[1]. Acetaminophen or paracetamol, as it is commonly known, is frequently used as an analgesic (pain reliever) and antipyretic (fever reducer) [2]. Combination of two or more drugs in pharmaceutical formulations is used in multiple theories [3]. Acetaminophen is used in combinations with different opioids in the treatment of post-surgery pains [2, 4]. Acetaminophen can be determined using various methods such as: optic methods [1-9], electro analytical methods [10-12] or chromatographic methods [13-15] and so on. Among the separation methods used for separation of drugs active principles we can mention membrane techniques [16-20]. Although there are a large number of articles that treat the subject of paracetamol determination and separation they do not refer to a

[1] PostDoc Student, Lecturer, Department of Analytical Chemistry and Environmental Engineering University POLITEHNICA Bucharest, Romania; E-mail: diaconuioana12@yahoo.com

[2] PhD Student, Department of Analytical Chemistry and Environmental Engineering University POLITEHNICA Bucharest, Arcuda Laboratory, Water Quality Service, Joita, Giurgiu, Romania, E-mail: criss_mirea2010@yahoo.com

[3] Reader, Department of Analytical Chemistry and Environmental Engineering University POLITEHNICA Bucharest, Romania; E-mail: e_ruse@chim.upb.ro

[4] student eng., Department of Analytical Chemistry and Environmental Engineering University POLITEHNICA Bucharest, Romania; E-mail: b.andreea@hotmail.com

major part of quality assurance namely the estimation of uncertainty in spectrophotometric determination of paracetamol.

The assessment of uncertainty associated with an analytical method is essential for demonstrating the quality of a result [21]. The result of a measurement is very important to be valid and determined in conditions well defined. The result is crucial in activities such as: commerce, legal actions or publications [22, 23]. The result of a measurement is just an estimation of the measuring. The measured value is accepted only when it is accompanied by a quantitative affirmation of the uncertainty [24, 25]. The uncertainty of the measurement is a useful tool in assessing the conformity or non-conformity during or at the end of the pharmaceutical process or in the evaluation of stability tests for pharmaceutical products [6, 7]. Uncertainty estimation is an essential condition for testing laboratories in order to obtain accreditation for an analytical research laboratory. Identification of uncertainty components and reasonable estimation are necessary for a statistical valid calculation for uncertainty estimation [5].

For uncertainty estimation the known approaches are top-down and bottom-up. The top-down approach mentions uncertainty can be estimated from reproducibility studies. The opposite is the approach bottom-up which quantifies uncertainty by summing the variance as presented in equation (1).

$$u_c(M) = M\sqrt{(\frac{u(a)}{a})^2 + (\frac{u(b)}{b})^2 + (\frac{u(c)}{c})^2 + (\frac{u(d)}{d})^2} \quad (1)$$

for a measurand M (analytical result), if the equation for M is based on multiplication and divisions like M= (a x b x c)/d [2].

Uncertainty in spectrophotometric determinations was studied by many scientists [28-30]. According to their results, physical sources of uncertainty often have significantly lower contributions than chemical sources. The calibration equations also have a significant contribution to the uncertainty in UV–Vis spectrophotometric analysis [28, 29]. Another study presents that the contributions of precision, linearity and weight of linezolid reference standard are the most significant, contributing with about 77% of the overall uncertainty. The Eurachem procedure was also compared to Monte Carlo simulation results. The conclusion was that the Eurachem procedure can be considered reasonable for the estimation of measurement uncertainty of linezolid by UV spectrophotometry.

In the present study a bottom-up approach is used to estimate the uncertainty of the determination of acetaminophen using UV-Vis spectrophotometry after its transport through bulk liquid membrane.

2. Experimental part

2.1. Reagents

All the reagents were analytical grade. The acetaminophen standard was purchased from Hebei Jiheng (Group) Pharmacy Co., Ltd.-China. Chloroform and tributhyl phosphate was purchased from Merck while NaOH was purchased from Fluka.

2.2. Material and equipment

The transport through the bulk liquid membrane takes place in a wall in wall type of cell presented in previous papers [31-34]. The membrane system consists from a feed phase -20 cm^3 10^{-1} mol/L acetaminophen-, membrane- 50 cm^3 10^{-1} mol/L tributhyl phosphate in chloroform- and stripping phase- 1 mol/L solution of NaOH. The transport time was of 24 h at a stirring speed of 180 rot/min.

The analytical control was realized using a LAMDA UV-VIS-NIR (Perkin Elmer Life and Analytical Sciences) spectrophotometer at acetaminophen specific wavelength at 241nm-for feed phase and 256 nm-for stripping phase.

2.3. Procedure for uncertainty estimation

Uncertainty due to concentration, c_{50}

The concentration of acetaminophen is obtained from a calibration curve established from a stock solution of acetaminophen 10^{-3} mol/L of acetaminophen resulted from the weighting 0.0151 g of acetaminophen and bringing them quantitatively to a 100 mL volumetric flask. 11 stock solution of acetaminophen are calculated with concentration ranging between $1 \cdot 10^{-5}$- $1{,}1 \cdot 10^{-4}$ mol/L in 1mol/L NaOH solution. All eleven solutions were measured three times. In this case the uncertainty of preparation of the stock solution is low enough to be neglected.

The uncertainty of the sample to be analyzed is obtained from equation (2)

$$u(c) = \frac{s_r}{b}\sqrt{\frac{1}{n} + \frac{1}{p} + \frac{(c - \bar{c})^2}{s_{xx}}} \tag{2}$$

where

$$s_r = \sqrt{\frac{\sum_{j=1}^{n}[Y_j - (bx_i + a)]}{n-2}} \quad (3)$$

s_r- residual standard deviation
n - number of measurements used for calibration curve
p - number of measurements used to obtain the concentration of the sample
c - analyte concentration in the unknown sample, mol/L
$\bar{c}$ - average of standard solution, mol/L

$$s_{xx} = \sum (c_i - \bar{c})^2 \quad (4)$$

where Y_j - analytical signal of the measurement j

j - analytical signal of the measurement j
i - index for the number of solution for the calibration
b – calibration curve slope, L/mol
a – calibration curve intercept
x_i – analyte concentration from standard solution mol/L
n – number of measurements made in order to obtain the calibration curve

The concentration of the sample from the stripping solution was obtained from the calibration curve which has a general formula presented in equation (5).

$$y=a+bx \quad (5)$$

where y-analytical signal, absorbance
a – calibration curve intercept
b – calibration curve slope, L/mol
x – analyte concentration, mol/L

Uncertainty of the discharge of 50 mL volumetric flask

The uncertainty in case of the repeatability of the discharge of the 50 mL volumetric flask was realized by using an experiment consisting in filling up and weighting a typical 50 mL volumetric flask with standard solution for ten times.

4. Results and discussions

The transport through bulk liquid membranes due to the presence of the carrier is very selective. Thus after the transport the acetaminophen an efficiency of 95.4% is obtained. The uncertainty of this quantity is than estimated.

In order to estimate correctly the uncertainty a scientist must understand correctly the process in order to present a correct equation of the measurand. Equation (6) presents the determination of acetaminophen after the transport.

$$Q = c_{50} \cdot V_{50} \cdot M_{\text{acetamin.}} \cdot F \cdot 10^{-3} \tag{6}$$

where Q- sample quantity, g

c_{50}- concentration of acetaminophen of the 50 mL volumetric flask, mol/L

V_{50}-volume of the volumetric flask, 50 mL

$M_{acetamin.}$-molecular mass of acetaminophen, g/mol

F-factor=1 responsible for spectrophotometer uncertainty, obtained from calibration certificate

Uncertainty due to concentration, c_{50}

After measuring the absorbance of the standard solution the absorbance values are obtained and presented in table 1.

The calibration curve is described by equation (5) and the result of the linear fitting is presented in table 2.

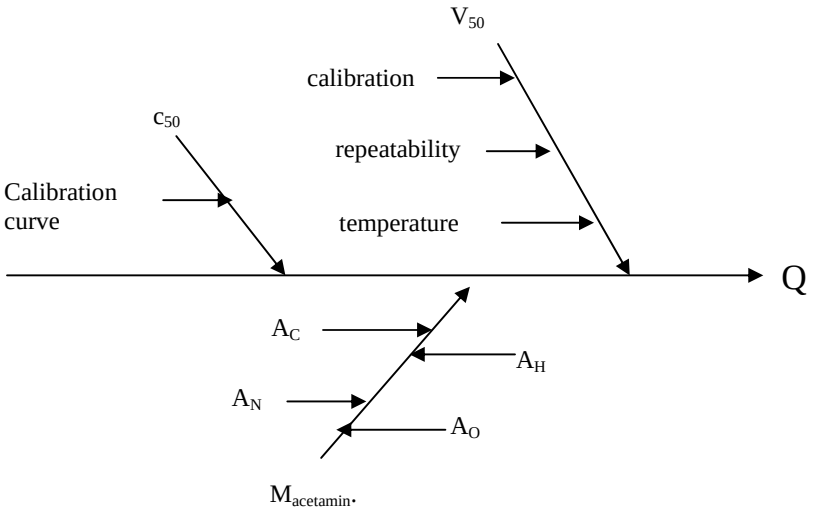

Fig. 1. The cause-effect diagram for the determination of acetaminophen through UV-Vis spectrophotometry after the transport through bulk liquid membrane

Table 1

Calibration data for acetaminophen

i	Concentration mol/L	A_1	A_2	A_3	$\overline{A}$
1	$1 \cdot 10^{-5}$	0.1014	0.1009	0.1012	0.1011
2	$2 \cdot 10^{-5}$	0.2133	0.214	0.2133	0.2135
3	$3 \cdot 10^{-5}$	0.3183	0.3181	0.3184	0.3182
4	$4 \cdot 10^{-5}$	0.4226	0.4233	0.424	0.4233
5	$5 \cdot 10^{-5}$	0.5367	0.536	0.536	0.5362
6	$6 \cdot 10^{-5}$	0.6443	0.6442	0.6442	0.6442
7	$7 \cdot 10^{-5}$	0.7406	0.7387	0.7399	0.7397
8	$8 \cdot 10^{-5}$	0.8592	0.8612	0.8615	0.8606
9	$9 \cdot 10^{-5}$	0.9431	0.9438	0.9441	0.9436
10	$10 \cdot 10^{-5}$	1.067	1.0707	1.0717	1.0698
11	$11 \cdot 10^{-5}$	1.1649	1.163	1.169	1.1656

The test solution was measured 10 times (p=10) and thus the concentration c_{50} was obtained. The results are presented in table 3.

After realizing the average of the standard solution concentration and average value is obtained=$6 \cdot 10^{-5}$mol/L.

Knowing the calibration curve equation y=10637.9697x-0.00044 we can identify the slope as b=10637.9697 L/mol and the intercept as a=-0.00044. For the determination of the calibration curve a number of 11 solution was measured, thus n=33.

Table 2

Linear fitting parameters for acetaminophen determination through UV-Vis spectrophotometry

Statistical parameter	Value
Calibration curve equation	y=10637.9697x-0.00044
Calibration curve slope, b	10637.9697
Standard deviation of the calibration curve slope, s_b	67.2439
Calibration curve intercept at the origin, a	-0.00044
Standard deviation of the calibration curve intercept at the origin, s_a	0.0045
Correlation coefficient, R	0.9998
Determination coefficient,R^2	0.999
Number of freedom degrees, n	9

Table 3

Sample determination for acetaminophen

i	Absorbance	Concentration, mol/L
1	0.4054	$3.81509 \cdot 10^{-5}$
2	0.4055	$3.81603 \cdot 10^{-5}$

3	0.4056	$3.81697 \cdot 10^{-5}$
4	0.4057	$3.81791 \cdot 10^{-5}$
5	0.4053	$3.81415 \cdot 10^{-5}$
6	0.4049	$3.81039 \cdot 10^{-5}$
7	0.4047	$3.80851 \cdot 10^{-5}$
8	0.4056	$3.81697 \cdot 10^{-5}$
9	0.4055	$3.81603 \cdot 10^{-5}$
10	0.4055	$3.81603 \cdot 10^{-5}$
Average		$3.81481 \cdot 10^{-5}$

Thus:

$$s_{xx}=1.1 \cdot 10^{-8} mol^2/L^2 \quad (6)$$

$$s_r=7.94 \cdot 10^{-9} \quad (7)$$

$$u(c)=3{,}1127 \cdot 10^{-13} mol/L \quad (8)$$

Thus the uncertainty associated with the concentration is $3{,}1127 \cdot 10^{-13}$mol/L and the standard uncertainty is:

$$\frac{u(c)}{c}=\frac{3.11 \cdot 10^{-13} mol/L}{3.81 \cdot 10^{-5} mol/L}=8.1595 \cdot 10^{-9} \quad (9)$$

Uncertainty associated with discharge of 50 mL volumetric flask

The volume has 3 major influences: calibration, repeatability and temperature effect. Other studies showed that another source of uncertainty is the aging of the volumetric instruments. This thing happens only if a strong base solution is preserved for a longer period of time. In our case this thing does not happen.

a. Calibration for the volumetric flask. The manufacturer offers for the volumetric flask a tolerance of ±0.05 mL at a temperature of 20 ºC. This uncertainty is not associated with a confidence range or other information regarding its distribution. The manufacturer uses a constant temperature and thus a triangular distribution is used in order to maintain a nominal value for the volume [22]. The uncertainty associated with the volume of volumetric flask due to calibration is presented in equation (10).

$$u(V_{cal})=\frac{0.05}{\sqrt{6}}=0.020412 \quad (10)$$

b. Repeatability. After the filling and weighting the volumetric flask a standard deviation of the measurement 0.01mL is obtained. This value can be used directly as standard uncertainty.

c. Temperature. According to the manufacturer the volumetric flask is calibrated at 20ºC, while in the laboratory the temperature varies with ±4ºC. The uncertainty due to this effect can be calculated from the estimation of the temperature range and the volume expansion coefficient which for water is $2.1 \cdot 10^{-4}$ ºC. The variation of the volume in this case is presented in equation (11).

$$\pm(50 \cdot 4 \cdot 2.1 \cdot 10^{-4}) = \pm 0.0042 \quad (11)$$

The temperature has a rectangular influence (if there aren't sufficient temperature data) or, as in the present case, a triangular distribution (the laboratory is equipped with air condition) [22]. Thus the uncertainty due the temperature effect is presented in equation (12).

$$u(V_{temp}) = \frac{0.042}{\sqrt{6}} = 0.017146 \quad (12)$$

The three contributions give the standard uncertainty of the volume V, namely u(V) presented in equation (13).

$$u(V_{50}) = \sqrt{u(V_{cal})^2 + u(V_{rep})^2 + u(V_{temp})^2} = 0.028472 \quad (13)$$

The standard relative uncertainty due to volume will be presented in equation (14).

$$\frac{u(V_{50})}{V_{50}} = 5.6944 \cdot 10^{-4} \quad (14)$$

Uncertainty due to the molar mass of acetaminophen

According to IUPAC [35] the elements atomic weights presents a certain uncertainty. In table 4 the element atomic weight of the elements involved in the acetaminophen formula and their uncertainty are presented. According to Eurachem Guide [36, 37] for standard uncertainty associated to the atomic weight of the elements a rectangular distribution is admitted. Thus the standard uncertainty is obtained by dividing these values to $\sqrt{3}$.

Table 4

Elements atomic weights and their standard uncertainty

Element	Atomic weight	IUPAC Uncertainty	Standard Uncertainty
C	12.0107	0.0008	$4.6188 \cdot 10^{-5}$
H	1.00794	0.00007	$4.04145 \cdot 10^{-5}$
N	15.994	0.00003	$1.73205 \cdot 10^{-5}$
O	14.000674	0.00007	$4.04145 \cdot 10^{-5}$

The molecular mass is the sum of the atomic weights and thus the uncertainty due to the molecular mass is obtained by summing the squares of the individual standard uncertainty [22, 36]. Thus the molecular mass of acetaminofen is 151.1457 g/mol and has a standard uncertainty associated with $u(M_{acetamin.})=1.3788 \cdot 10^{-5}$.

The relative standard uncertainty associated with the molecular mass is presented in equation (15).

$$\frac{u(M_{\text{acetamin.}})}{M_{\text{acetamin.}}} = 9.1226 \cdot 10^{-8} \tag{15}$$

The intermediate values, their standard uncertainty and their relative standard uncertainty are synthesized in table 5.

Table 4

Uncertainty sources and their standard and relative uncertainty at the determination of acetaminophen determination through UV-Vis spectrophotometry

Parameter	Value	Standard uncertainty, u(x)	Relative standard uncertainty, u(x)/x
Volume, V_{50},mL	50	$2.84 \cdot 10^{-2}$	$5.6944 \cdot 10^{-4}$
Sample concentration, c_{50}, mol/L	$3.8148 \cdot 10^{-5}$	$3.1127 \cdot 10^{-13}$	$8.1595 \cdot 10^{-9}$
Molecular mass, $M_{acetamin.}$	151.145	$1.3788 \cdot 10^{-5}$	$9.1226 \cdot 10^{-8}$
F	1	$2.49 \cdot 10^{-8}$	$1.2437 \cdot 10^{-8}$

In order to determine the composed uncertainty of the sample quantity Q is obtained using equation 16.

$$\frac{u(Q)}{Q} = \sqrt{\left(\frac{u(V_{50})}{V_{50}}\right)^2 + \left(\frac{u(c_{50})}{c_{50}}\right)^2 + \left(\frac{u(M_{\text{acetamin.}})}{M_{\text{acetamin.}}}\right)^2 + \left(\frac{u(F)}{F}\right)^2} \tag{16}$$

Thus $Q=2.8829 \cdot 10^{-4}$g and its standard uncertainty is $u(Q)=1.6416 \cdot 10^{-7}$g.

Considering a confidence level of 95 % and a coverage factor k=2 the expanded uncertainty is $U(Q)= 3.2833 \cdot 10^{-7}$g.

6. Conclusions

The present study illustrates a cause-effect analysis for estimating the uncertainty in a usual determination of acetaminophen determination through UV-Vis spectrophotometry. The acetaminophen is resulted at the transport through bulk liquid membranes. A major contribution to the uncertainty budget is represented by the discharge of the 50 mL volumetric flask.

Acknowledgement

The work has been funded by the Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132395.

R E F E R E N C E S

[1]. *M. E. Bosch, A. J. Sanchez, F. S. Rojas and C. B. Ojeda*, Determination of paracetamol: Historical evolution, J. Pharm. Biomed. Anal., **vol.42**, no.3, 2006, pp. 291-321

[2]. *S. Behera S. Ghanty, F. Ahmad, S. Santra and S. Banerjee,.* UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation J.Anal.Bianal.Tech., **vol.** 3, no. 6, 2012.

[3]. *M. S. Kondawar, R. R. Shah, J. J. Waghmare, N. D. Shah, and M. K. Malusare*, UV spectrophotometric estimation of Paracetamol and Lornoxicam in bulk drug and tablet dosage form using multi-wavelength method, Intl. J. PharmTech Res., **vol.** 3,no.3, 2011, pp. 1603-1608

[4]. Scottish Intercollegiate Guidelines Network (SIGN) (2008) Control of pain in adults with cancer-A national clinical guideline. Scotland: National Health Service (NHS).

[5]. *A. C. Moț, F. Soponar, A. Medvedovici, and, C. Sârbu,* Simultaneous spectrophotometric determination of aspirin, paracetamol, caffeine, and chlorphenamine from pharmaceutical formulations using multivariate regression methods, Anal. Lett., **vol.** 43, no. 5, 2010, pp. 804-813.

[6]. *M. K. E. Al-Shwaiyat*, Spectrophotometric Determination of Paracetamol by Reduction of 18-Molybdo-2-Phosphate Heteropoly Anion, Jordan J.Chem., **vol.8**, no.2, 2013, pp. 79-89

[7]. *B. R. Shrestha, and R. R. Pradhananga*, Spectrophotometric method for the determination of paracetamol , J. Nepal Chem.Soc., no. *24*, 2009, pp.39-44.

[8]. *M. L. Ramos, J. F. Tyson, and D. J. Curran*, Determination of acetaminophen by flow injection with on-line chemical derivatization: Investigations using visible and FTIR spectrophotometry , Anal.Chim. Acta, **vol. 364**, no. 1, 1998, pp. 107-116.

[9]. *S. D. Cekic, H. Filik, and R Apak*, Simultaneous spectrophotometric determination of paracetamol and p-aminophenol in pharmaceutical products with tiron using dissolved oxygen as oxidant , J.Anal. Chem., **vol. 60**, no. 11, 2005, pp. 1019-1023.

[10]. *X. Kang, J. Wang, H. Wu, J. Liu, I. A. Aksay and Y. Lin*, A graphene-based electrochemical sensor for sensitive detection of paracetamol , Talanta, **vol.** 81, no.3, 2010, pp. 754-759.

[11]. *M. Mazloum-Ardakani, M. A. Sheikh-Mohseni, M. Abdollahi-Alibeik and A. Benvidi,* Electrochemical sensor for simultaneous determination of norepinephrine, paracetamol and

folic acid by a nanostructured mesoporous material , Sens. Actuators, B, no.171, 2012, pp. 380-386.

[12]. *N. F., Atta, A. Galal, F. M. Abu-Attia, and S. M Azab*. Simultaneous determination of paracetamol and neurotransmitters in biological fluids using a carbon paste sensor modified with gold nanoparticles , J. Mat. Chem., **vol.** 21, no. 34, 2011, pp. 13015-13024.

[13]. *B. Tsvetkova, I. Pencheva, A. Zlatkov, and P. Peikov.* Simultaneous high-performance liquid chromatography determination of paracetamol and ascorbic acid in tablet dosage forms , African J. Pharmacy and Pharmacology, **vol. 6,** no. 17, 2012, pp. 1332-1336.

[14]. *E, Deconinck, P. Y. Sacre, S. Baudewyns, P. Courselle and J. De Beer* (2011). A fast ultra high pressure liquid chromatographic method for qualification and quantification of pharmaceutical combination preparations containing paracetamol, acetyl salicylic acid and/or antihistaminics , J. Pharm.Biomed.Anal., **vol. 56,** no. 2, 2011, pp. 200-209.

[15]. G. *Ramanaiah, D. Ramachandran, G. Srinivas, G. Jayapal, P. Rao, and V Srilakshmi.* Development and Validation of Stability Indicating RP-LC Method for Simultaneous Estimation of Tapentadol and Paracetamol in Bulk and its Pharmaceutical Formulations. , Drug Invention Today, **vol. 4,** no 7, 2012, pp. 391-396.

[16]. *I. Diaconu, I. Zaharia. E. Ruse and D. A. Radu,* Kinetic Aspects of Transport para-aminophenol through Agitated Bulk Liquid Membrane , Rev. Chim., **vol. 63,** no. 2, 2012, pp. 153-158.

[17]. *I. Zaharia, I. Diaconu , E. Ruse and G. Nechifor*, Transport and separation through bulk liquid membrane of some biologic active compounds. , Analele Universitatii" Ovidius" Constanta-Seria Chimie, **vol. 23,** no. 1, 2012, pp. 53-57.

[18]. I*. Zaharia, I. Diaconu, E. Ruse, and G. Nechifor,* The transport of 3-aminophenol through bulk liquid membrane in the presence of ALIQUAT 336 , DIG J NANOMATER BIOS, **vol. 7,** no. 3, 2012, pp. 1303-1314.

[19]. *I. Zaharia, I. Diaconu, G Nechifor*, The pH role in the transport of active principles of drugs through agitated bulk liquid membrane , U.P.B. Sci. Bull., Series B, **vol. 74,** no. 2, 2012, pp. 61-70

[20]. *I. Zaharia, H. Y. Aboul-Enein, I. Diaconu, E. Ruse, A. A. Bunaciu and G. Nechifor* Facilitated transport of 5-aminosalicylic acid through bulk liquid membrane , J.Iran.Chem.Soc., **vol. 10,** no.6, 2013, pp. 1129-1136.

[21]. *Ó. Pindado Jiménez, and R. M. Pérez Pastor,* Estimation of measurement uncertainty of pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons in sediments by using gas chromatography–mass spectrometry , Anal.Chim.Acta, no. *724*, 2012, pp. 20-29

[22]. *V. R. Meyer*, Measurement uncertainty , J.Chromatogr. A, **vol. 1158,** no. 1, 2007, pp. 15-24.

[23]. Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, 1995, ISBN 92-67-10188-9.

[24]. *A. Elisavet, T. Eleni, T. Lazaros and K. Foteini*, Uncertainty in spectrophotometric analysis—"Error propagation break up", a novel statistical method for uncertainty management , Talanta, **vol. 85,** no. 5, 2011, pp. 2385-2390.

[25]. ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories

[26]. M*. A. L. Traple, A. Saviano, F. L. Francisco and F. R. Lourenço,* Measurement uncertainty in pharmaceutical analysis and its application , J. Pharm. Anal., **vol. 4,** no. 1, 2014, pp. 1-5

[27]. *I. Diaconu, H. Y. Aboul-Enein, A. A. Bunaciu, and I. G. Tănase,* Estimation of uncertainty for measuring codeine phosphate tablets formulation using UV-VIS spectrophotometry , Anal. Lett.*,* **vol. 43,** no. 7-8, 2010, pp. 1207-1216

[28]. *L. Sooväli, E. I. Rõõm, A. Kütt, I. Kaljurand, and, I. Leito,* Uncertainty sources in UV-Vis spectrophotometric measurement , ACCREDIT QUAL ASSUR, **vol. 11,** no. 5, 2006, pp. 246-255

[29]. *K. H. Hsu, and C. Chen*, The effect of calibration equations on the uncertainty of UV–Vis spectrophotometric measurements , Measurement, **vol. 43,** no. 10, pp. 1525-1531.

[30]. *A. M. Saviano, and F. R. Lourenço*, Uncertainty evaluation for determining linezolid in injectable solution by UV spectrophotometry , Measurement, **vol. 46,** no. 10, 2010, pp. 3924-3928.

[31]. Josceanu, A.M., Ruse, E., Luca, C., Cerbu, E. Optimization of the transport of aminic substrates, Mat. Sci. Eng.C, **vol 18** , no 1-2, 2001, pp. 79-85

[32]. *I. Diaconu, H.Y. Aboul-Enein, M.A. Al-Omar, G. Nechifor, E. Ruse, A..A. Bunaciu, E.E Totu.* Separation of nitrophenols. Equilibriums in bi- and tri-phasic systems Arab. J.Chem., **vol. 4,** no. 1, 2011, pp. 99-103

[33*]. I. Diaconu, G. Nechifor, A.C. Nechifor, E. Ruse, E.E. Totu,* Membranary techniques used at the separation of some phenolic compounds from aqueous, UPB.Sci.Bull. Serries B**,**, **vol. 71,** no. 4, 2009, pp. 61-70

[34]. *I. Diaconu, G. Nechifor, A.C. Nechifor, E.E Totu, E. Ruse,* The transport of nitrophenols through liquid membranes, Rev. Chim. **vol. 60,** no. 12, 2009, pp. 1243-1246.

[35]. J*. R. de Laeter, J. K. Böhlke, P. De Bièvre, H. Hidaka, H. S. Peiser, H. S., K. J. R. Rosman, P. D. P .Taylor*,. Pure Appl. Chem., Atomic weights of the elements. Review 2000 (IUPAC Technical Report). **Vol. 75**, no 6, 2003, pp. 683-800.

[36] Quantifying Uncertainty in Analytical Measurement, 2nd Edition (2000)

[37] Quantifying Uncertainty in Analytical Measurement, 3rd Edition