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Development and Validation of Sequential Injection Amperometric System for Analysis of Thiopurine Antimetabolic Drugs

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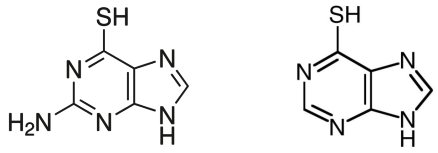
Abstract

Utilizing the methodology of sequential injection (SI) analysis, a novel automated method was developed for the rapid determination of 6-thioguanine (6-TG) and 6-mercaputopurine (6-MP) which are well-known antimetabolic drugs. The method involved a direct amperometric detection of these compounds by using an electrochemically activated carbositall electrode (CSE). The linear dynamic ranges of 0.1 – 100 and 0.2 – 200 μg mL^{-1}, with the detection limits (S/N =3) of 0.03 and 0.06 μg mL^{-1}, were obtained for 6-TG and 6-MP, respectively. Sampling rate – 70 h^{-1}, sample volume – 500 μL. The applicability of the proposed SI-amperometric system was demonstrated by analysing the thiopurine drugs with good recoveries (98.8 – 102.0 %) and reproducibility of the electrode response (RSD < 2.0 %). The method can be useful for automated dissolution testing of thiopurine antimetabolic drugs.

Keywords Sequential injection analysis, thiopurine antimetabolic drugs, amperometric detection, activated carbositall electrode

1. Introduction

Thiol-containing drugs are widely used for the treatment of many diseases. In particular, sulphur containing structural analogues of purine bases such as 6-thioguanine (6-TG, 2-amino-1H-purine-6(7H)-thione) and 6-mercaptopurine (6-MP, 3,7-dihydropurine-6-thione) are most commonly used as cytostatic agents in chemotherapy for the treatment of leukaemia and in therapy of inflammatory diseases such as ulcerative colitis, dermatitis and some other pathology (Fig.1) [1–3]. The cytotoxic activity of 6-MP is affected by thiopurine methyltransferase (TPMT), a genetically regulated and variable intracellular enzyme. 6-TG, a closely related thiopurine, is less affected by the enzyme and so it may be a more reliable drug-at least for patients with constitutionally high TPMT activity.



6-TG 6-MP

Fig. 1 Molecular structures of 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP).

Successful exploitation of thiopurine drugs requires the regular control of their concentration in both drug formulations and body fluids. To date, several analytical procedures have been proposed for the determination of thiopurines in different samples by using spectrophotometry [4–6] and spectrofluorimetry [7–10]. A number of researchers have reported electrochemical determination of thiopurine drugs utilizing different types of electrodes, mainly chemically modified electrodes (CMEs) [5, 11–22]. Given the use of a large number of cytotoxic preparations per day, simple and fast quantitative measurements are of particular importance. Therefore, a lot of attention is given to use of flow-based analysis techniques [23].

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High-performance liquid chromatography [24–29] and capillary electrophoresis [30, 31] coupled with various detector techniques such as UV- and mass-spectrometry, amperometry and laser induced fluorescence have been proposed for the determination of thiopurines. An integrated microfluidic device with on-line labelling, electrophoresis separation and chemiluminescence detection have been developed for the simultaneous quantification of thiol-containing drugs, including 6-TG and 6-MP [32]. However, these methods show their limitations and demerits in practical application. In particular, selection of a suitable mobile phase and/or finding a suitable reactant for post-column reaction is a rather complicated task. In addition, most of these methods are time-consuming and not sensitive enough to allow determining thiopurines in small aliquots while they also depend on expensive tools for screening.

The methodology of flow injection (FI) analysis and its alternative approach such as sequential injection (SI) analysis would appear to be suitable for the analytical control of both drug formulations and body fluids [33 – 37]. To date, FI systems for the chemiluminescence detection of thiol-containing drugs have been developed [38–41]. In contrast, the potential of electrochemical detection of thiopurines in FI or SI systems has not yet been report so far. Nevertheless, such approach is seems to be very promising from practical point of view [42].

The objective of the current study was to develop and validate a novel automated method for the quantitation of thiopurines (6-TG, 6-MP) in pharmaceutical formulations by using sequential injection amperometric (SI-Amp) system with an electrochemically activated carbositall electrode (CSE). Carbositall is a synthetic pyrocarbon material that consists of the nanocarbon phase containing large and small monocrystalline inclusions of boron (4 – 5 % mass.). The remarkable properties of this material (*e.g.* high surface volume ratio, high electrical conductivity, wide potential window in the positive range, chemical inertness and strong mechanical strength) created interest in its electrochemical applications [43]. The suitability of CSE with the pre-activated surface for the electrochemical measurements under hydrodynamic conditions was demonstrated in our previously published works [43, 44]. In particular, such

electrode was successfully used for the flow-injection amperometric determination of antiviral guanine derivatives in different pharmaceutical forms [44].

2. Experimental

2.1 Reagents

Thiopurines (6-TG and 6-MP) and all other chemicals were purchased from Across Organics or Sigma-Aldrich and used without any further purification. Stock solutions of purines (1.0 mmol L^{-1} and 1.0 mg L^{-1}) were prepared by dissolving precisely weighed amounts of the pure substances with 5.0 mmol L^{-1} NaOH. All solutions were stored at +4°C in the dark at least one week. Working standard solutions were freshly prepared by stepwise diluting the respective stock solutions with a phosphate buffer solution (PBS) as supporting electrolyte. PBSs with various pH values were prepared by mixing of 0.1 mol L^{-1} NaH_2PO_4, 0.1 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} H_3PO_4 and 0.1 mol L^{-1} KCl. Doubly distilled water was used throughout all the experiments. The solutions were deoxygenated by passing nitrogen gas.

2.2 Apparatus

An automatic FIALab 3500 analyzer with a programmable flow (FIAlab Instruments, Inc., WA., USA) was used for hydrodynamic voltammetric measurements and amperometric detection of thiopurines. The electrochemical measurements were performed with an Ecotest-VA analyzer (Econix-Expert, Russia) interfaced to a computer system with MDEV software. A three-electrode flow cell containing a carbositall dick working electrode (Volta, Russia), an Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode and a platinum wire auxiliary electrode was used. All potentials are referred to Ag/AgCl electrode at 25 ± 1°C. Schematic illustration of the SI-Amp system is shown in Fig. 2.

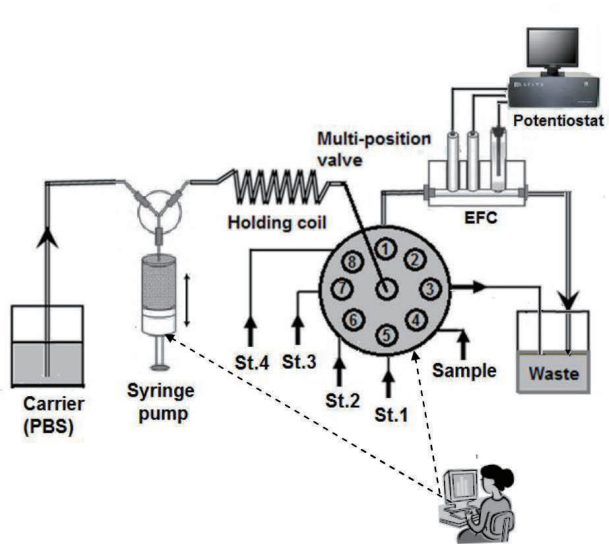

Fig. 2 Schematic diagram of the SI-Amp system.

The computer program Origin 6.1 based on the Levenberg-Marquardt algorithm was used for signal processing and peak analysis. pH values were tested by using a pH-meter Model OP-110 (Radelkis, Hungary). The ultrasonic bath Elmasonic One (Germany) with 35-kHz ultrasound was used for irradiation of the CSE surface.

2.3. Working carbositall electrode activation

Prior to activation, the CSE surface was polished with 0.3 and 0.05 μm alumina powders to mirror like. The polished electrode was then rinsed with water, and ultrasonically irradiated subsequently in anhydrous ethanol for 1 min and doubly distilled water for 30 s. The pre-treated CSE was electrochemically activated in 0.1 mol L^{-1} $HClO_4$ by scanning potential in the range of (0.0 – +1.5) V for 20 cycles (voltage scan rate v = 0.05 V s^{-1}). Further, this electrode was scanned between -1.0 and +1.4 V in 0.1 mol L^{-1} PBS until a steady-state current-voltage curve was obtained. After the activation, the surface layer of carbositall has a highly porous structure with different pore diameters and dispersed inclusions [43]. As a result, the active surface area of the electrode increased from 7.1 mm^2 to 12.8 mm^2. The activated CSE was stored in contact with air at the room temperature.

2.4 Operating procedure

The protocol established for the amperometric detection of thiopurines is given in Table 1 and comprised five steps. The analytical cycle consisted in a calibration of electrode, followed by amperometric detection of one or more samples.

Table 1 Protocol for the detection of thiopurines using SI-Amp system

Step	Action	Valve position	Flow rate (μLs^{-1})	Volume (μL)
1	Fill syringe barrel with carrier (PBS)	–	300	1800
2	Flush and fill EFC with the PBS (back-ground electrolyte)	1	200	1000
3	Aspirate sample (or standard solutions) into holding coil	4 (5–8)	50	500
4	Zone flushing through EFC for detection	1	30	900
5	Refill syringe barrel	–	300	1800

2.5 Preparation and analysis of pharmaceutical samples

All pharmaceuticals were collected from a local drugstore. For analysis, five commercial tablets were accurately ground and homogenized. Then, an appropriate amount of the powder was ultrasonically shaked in 100 ml of 0.5 mmol L^{-1} NaOH for about 15 min. Then, the obtained solution was centrifuged for 10 min and filtered on a 0.45 μm filter into a volumetric flask with an appropriate volume. The treated solution was used as a stock solution for the experiments. The working sample solutions were prepared by diluting the stock solutions with 0.1 mol L^{-1} PBS of pH ~ 2 – 7.

3. Results and Discussions

3.1 Preliminary study on voltammetric behaviour of thiopurines at the activated CSE

According to the literature, electrochemical detection of thiopurines was often hampered by the slow electron transfer kinetics at the electrode surface, so that the sensitivity for determining 6-MP and 6-TG was very low. In particular, the linear and cyclic sweep voltammetry of thiopurines in solutions indicated that these compounds exhibited very small redox-peaks at the inactivated CSE. It was found, however, that the anodic activation of this electrode surface remarkably reduced the electrochemical signals and improved the electron transfer rates of the corresponding redox reactions (Fig. 3).

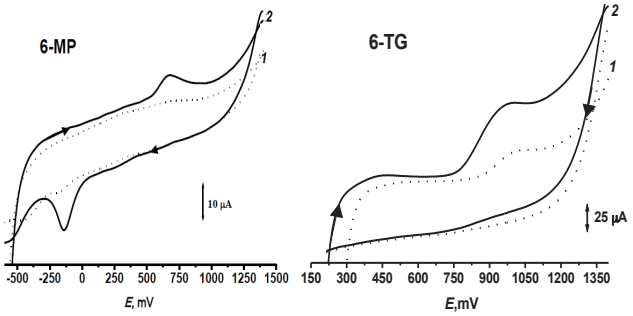

Fig. 3 Cyclic voltammograms of 6-MP (pH 1.7; 0.05 V s^{-1}) and 6-TG (pH 5.9; 0.1 V s^{-1}) at the activated CSE.

As it can be seen, a pair of well-defined redox peaks was clearly observed on the cyclic voltammogram obtained for 0.5 mmol L^{-1} 6-MP. In contrast, 6-TG exhibited a well-defined anodic peak, indicating that the electrode process of TG oxidation is an irreversible one. The oxidation behaviour of both thiopurines on the activated CSE was found to be dependent on the pH of the supporting electrolyte. The each peak potential shifted to the negative direction and background corrected peak currents decreased with increasing pH from 1.7 to 7.0, suggesting that protons are involved in the electrode reactions. Due to the literature, the investigated compounds exist in several tautomeric forms [45]. This appears to be reasonable to propose that the anodic peaks could be attributed to the oxidation of thiopurines when thio group exists as –SH (Fig. 1).

In order to get more information regarding the suitability of the activated CSE for quantitative measurements of 6-TG and 6-MP, hydrodynamic amperometry of these compounds (under laminar flow conditions) was performed using a SI-Amp system shown in Fig. 2. An applied potential (E_{app}) on recorded signals (H) was evaluated. Fig. 4 shows the hydrodynamic voltammograms obtained for 500-µL injections of 0.1 mmol L^{-1} solutions of each thiopurine using 0.1 mol L^{-1} PBS as the carrier. The results showed that the presence of maximal signals for both compounds correlated with the data obtained from cyclic voltammetry. The current response of the activated CSE under hydrodynamic conditions does not change by more than 4 % during 4 weeks.

3.2 Optimized parameters and analytical performance of the SI-Amp system

On the basis of the above mentioned results, the SI-Amp system for the direct amperometric detection of thiopurines at the activated CSE was developed. The choice of the best operation parameters of the system was made by considering the criteria of highest signal shape and lowest background current.

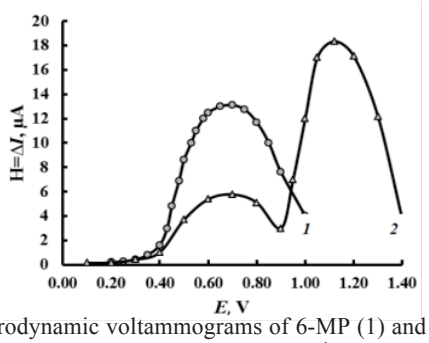

Fig. 4 Hydrodynamic voltammograms of 6-MP (1) and 6-TG (2) obtained at the activated CSE in 0.1 mol L^{-1} PBS of pH 2.3 (1) and 4.0 (2). Flow rate: 2.0 mL min^{-1}.

The influence of a flow rate and a sample volume on the peak height H under the fixed applied potential was evaluated. As it can be seen from Fig.5, the higher H was reached with the flow rate of 1.5 – 2.0 mL min^{-1}. The optimized value of the injected sample volume was found to be 500 µL.

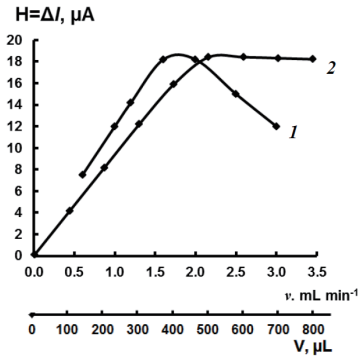

Fig. 5 Effect of varying flow rate (*1*) and sample volume (*2*) on the peak height H of 0.1 mmol L^{-1} solution of 6-TG.

Under the optimum condition chosen, a good linear relationship between H and the concentration of thiopurines (*c*) was obtained over a wide dynamic range. The characteristics of the calibration plots are listed in Table 2.

Table 2 Regression data of the calibration plots for the amperometric determining 6-TG and 6-MP by using the SI-Amp system

Criteria	Analytical characteristics			
	6-TG		6-MP	
pH	4.02		2.30	
Measured potential (V)	1.12 ± 0.01		0.65 ± 0.05	
Linear dynamic range (µg mL^{-1})	0 – 5	5–100	0–20	20–200
Slope	0.470	0.30	0.095	0.082
Intercept (µA)	0.005	0.62	0.002	0.069
Correlation coefficient (*r*)	0.9996	0.9999	0.9998	0.9996
Limit of detection (µg mL^{-1})	0.03	–	0.06	–
Limit of quantitation (µg mL^{-1})	0.1	–	0.2	–

In order to check the correctness of the new method, the recovery and reproducibility were evaluated for different concentrations of thiopurines (Table 3). The measurements gave excellent accuracy of current peaks and appreciable relative standard deviations (RSD).

The above results clearly indicate that the developed method is environmentally friendly and useful for the rapid and very convenient for the determination of thiopurines in anti-metabolic drugs.

The results showed that the analytical characteristics obtained for thiopurines by the proposed method, in particular limit of detection (LOD) and linear dynamic range, are comparable to, and even better than, those reported in the literature (Table 4).

Table 3 The recovery tests of thiopurines in standard solutions (n = 6, P=0.95)

Analyte	Added, μg mL^{-1}	Found, μg mL^{-1}	Recovery, %	RSD, %
6-TG	5.0	5.1 ± 0.1	102.0	1.9
	10.0	10.1 ± 0.2	101.0	1.9
	25.0	24.7 ± 0.4	98.8	1.5
	40.0	40.0 ± 0.6	100.0	1.4
6-MP	10.0	10.0 ± 0.2	100.0	2.0
	20.0	20.1 ± 0.3	100.5	1.4
	50.0	50.4 ± 0.7	100.8	1.3
	100.0	99.7 ± 0.9	99.7	0.9

Table 4 Comparison of analytical parameters of several flow-based methods developed for detecting thiopurines in pharmaceutical formulations and biological samples

Method	Analyte	Linear range, μM	LOD (3σ), μM	Recovery, %	Reference
Stopped-flow method / SPhD	6-MP	0.7 – 3.3	0.07	> 95	[6]
LC / ECD with NiHCNFe-modified GCE		1.0 – 100	0.5	–	[26]
HPLC / ECD with MWCNTs-modified GCE		0.4 – 400	0.2	98.0 – 102.0	[27]
CE / UV detector		1.0 – 20.0	0.1	77.08	[31]
CE / UV detector		33 – 330	6.58	98.3 – 101.3	[46]
FIA-CL / Luminol-H_2O_2 – gold colloid		0.04 – 7.0	0.001	93.4 – 105.3	[40]
FIA-CL / $KMnO_4$ - thioacetamide		0.005 – 0.66	0.0001	98.0 – 104.0	[41]
LC / ECD with Ag-electrode	6-TG	0.1 – 10.0	0.008	99.0 – 99.9	[29]
CE / UV detector		18 – 150	9.6	94.3 – 114.3	[47]
LC–MS/MS	6-TG	0.04 – 1.2	–	94.1 –106.9	[48]
	6-MP	0.04 – 1.3	–	97.2 – 108.3	
SI –Amp / activated CSE	6-TG	0.6 – 600	0.005	99.8 – 101.0	This work
	6-MP	1.0 – 1300	0.009	100.8 – 101.2	

LC – liquid chromatography; CE– capillary electrophoresis; MS/ MS– tandem mass spectrometry; ECD – electrochemical detection; SPhD – spectrophotometric detection; GCE – glassy carbon electrode; MWCNTs – multiwall carbon nanotubes.

3.3 Analysis of dissolved pharmaceutical formulations

To evaluate the applicability of the proposed method for practical use, 6-TG and 6-MP in commercial pharmaceutical formulation were determined. The recovery of the proposed procedure was evaluated by comparing the obtained results with those declared in the label of the pharmaceutical preparations (Table 5).

Table 5 The results of the determination of thiopurines in commercial dosage forms (n = 6, P = 0.95)

Dosage form	Analyte	Declared amount, mg	Found, mg	Recovery, %	RSD, %
Lanvis tablets	6-TG	40.0	40.4 ± 0.8	101.0	1.9
			39.9 ± 0.7	99.8	1.7
MP-tablets	6-MP	50.0	50.6 ± 0.9	101.2	1.7
			50.4 ± 0.8	100.8	1.5

The data given in Table 5 show the satisfactory results and indicate that Lanvis tablets matrix (inactive ingredients – lactose, potato starch, acacia, stearic acid and magnesium stearate) and Mercaptopurine tablets matrix (lactose, maize starch, stearic acid, magnesium stearate) did not cause appearance of any additional signals in examined potential window. The novel method seems to be very promising tool for automated dissolving testing of thiopurine anticancer tablets.

4. Conclusions

The use of electrochemical detectors in SIA systems has gained popularity in recent years due to some advantageous characteristics [49]. From the results of the present study it is apparent that the new automated SI-Amp method is very effective for rapid quantification of thiopurines within the framework of green analytical chemistry [50]. The electrochemical activity and response stability of the activated CSE to 6-TG and 6-MP provides high sensitivity and good reproducibility of amperometric measurements. The resulting procedure could be recommended for quality control of a large number of commercial anticancer drugs in an accurate, reproducible, quick and economic way.

Acknowledgments

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References

[1] D.E. Thurston, Chemistry and pharmacology of anticancer drugs. Boca Raton: CRC Press. Taylor and Francis Group (2007).
[2] J. Aarbakke, G. Janka-Schaub, G.B. Elion, *Trends Pharmacol. Sci*, **18**, 3 (1997).
[3] C.J. Cara, A.S. Pena, M. Sans, L. Rodrigo, M. Guerrero-Esteo, J. Hinojosa, J. García-Paredes, L.G. Guijarro, *Med. Sci. Monit.*, **210**, RA247 (2004).
[4] A.H. Chalmers, *Biochem. Med*., **12**, 234 (1975).
[5] J. Barek, *Talanta*, **32**, 987 (1985).
[6] J. Kurzawa, K. Janowicz, *Chem. Anal. (Warsaw)*, **48**, 741 (2003).
[7] A.D. Thomas, *Talanta*, **23**, 383 (1976).
[8] L. Wang, Z. Zhang, *Talanta*, **76**, 768 (2008).
[9] X.C. Shen, L.F. Jiang, H. Liang, X. Lu, L.J. Zhang, X.Y. Liu, *Talanta*, **69**, 456 (2006).
[10] M.X. Gao, J.L. Xu, Y.F. Li, C.Z. Huang, *Anal. Methods*, **5**, 673 (2013).
[11] Y. Sato,Y. Ishikawa, H. Matsuura, K. Uosaki, F. Mizutani, O. Niwa, *Electroanalysis*, **17**, 965 (2005).
[12] W. Wang, S.-F. Wang, F. Xie, *Sens. Actuat. B*, **120**, 238 (2006).
[13] B.-Y. Lu, H. Li, H. Deng, Z. Xu, W.-S. Li, H.-Y. Chen, *J. Elecroanal. Chem*., **621**, 97 (2008).
[14] E. Mirmomtaz, A.A. Ensafi, H. Karimi-Maleh, *Electroanalysis*, **20**, 1973 (2008).
[15] E. Mirmomtaz, A.A. Ensafi, *Electrochim. Acta*, **54**, 4353 (2009).
[16] A.A. Ensafi, H. Karimi-Maleh, *J. Electroanal. Chem*., **640**, 75 (2010).
[17] H. Beitollahi, J.-B. Raoof, R. Hosseinzadeh, *Anal. Sci*., **27**, 991 (2011).
[18] P. Zhou, L. He, G. Gan, S. Ni, H. Li, W. Li, *J. Elecroanal. Chem*., **665**, 63 (2012).
[19] S. Shahrokhian, F. Ghorbani-Bidkorbeh, A. Mohammadi, R. Dinarvand, *J. Solid State Electrochem*., **16**, 1643 (2012).
[20] M. Keyvanfard, V. Khosravi, H. Karimi-Maleh, K. Alizad, B. Rezaei, *J. Mol. Liq*., **177**, 182 (2013).
[21] H. Karimi-Maleh, A.F. Shojaei, K. Tabatabaeian, F. Karimi, S. Shakeri, R. Moradi, *Biosens. Bioelectron*., **86**, 879 (2016).
[22] M.K. Halbert, R. P. Baldwin, *Anal. Chim. Acta*, **187**, 89 (1986).
[23] G.D. Christian, *Anal. Chim. Acta*, **499**, 5 (2003).
[24] S. Nussbaumer, P. Bonnabry, J.-L. Veuthey, S. Fleury-Souverain, *Talanta*, **85**, 2265 (2011).
[25] E.C. Van Os, J.A. McKinney, B.J. Zins, D.C. Mays, Z.H. Schriver, W.J. Sandborn, J.J. Lipsky, *J. Chromatogr. B Biomed. Sci. Appl*., **679**, 147 (1996).
[26] S. Zhang, F. Huang, X. Cao, P. Yang, W. Zhang, L. Jin, Analyst, **127,** 485 (2002).
[27] X.N. Cao, L. Lin, Y.Y. Zhou, G.Y. Shi, W. Zhang, K. Yamamoto, L.T. Jin, *Talanta*, **60**, 1063 (2003).
[28] A.F. Hawwa, J.S. Millership, P.S. Collier, J.C. McElnay, *J. Pharm. Biomed. Anal*., **49**, 401 (2009).
[29] N. Karadas-Bakirhan, A. Sarakbi, M. Vandeput, S.A. Ozkan, J.M. Kauffmann, *Anal. Chem*., **87**, 6730 (2015).
[30] P. Lochman, T. Adam, D. Friedecky, E. Hlídková, Z. Skopková, *Electrophoresis*, **24**, 1200 (2003).
[31] C.-C. Wang, S.-S. Chiou, S.-M. Wu, *Electrophoresis*, **26**, 2637 (2005).
[32] Y. Huang, S. Zhao, M. Shi, H. Liang, *J. Pharm. Biomed. Anal*., **55**, 889 (2011).
[33] J. Ruzicka, G.D. Marshall, *Anal. Chim. Acta*, **237**, 329 (1990).
[34] J. Ruzicka, *Talanta*, http://dx.doi.org/10.1016/j.talanta.2017.08.061.
[35] Advances in Flow Injection Analysis and Related Techniques, S.D. Kolev, I.D. McKelvie (Eds.), Amsterdam: Elsevier (2008).
[36] P. Solich, M. Polášek, J. Klimundová, J. Ruzicka, *Trends Anal. Chem*., **22**, 116 (2003).
[37] P.D. Tzanavaras, *Open Chem. Biomed. Methods J*., **3**, 37 (2010).
[38] P. Viñas, I. Lopez Garcia, J.A. Martinez Gil, *J. Pharm. Biomed. Anal*., **11**, 15 (1993).
[39] D.-h. Zhao, Y.-q. Zi, *Chin. J. Anal. Lab*., **25**, 65 (2006).
[40] P. Yang, Y. Chen, Q. Zhu, F. Wang, L. Wang, Y. Li, *Microchim. Acta*, **163**, 263 (2008).
[41] L. Wang, B. Ling, H. Chen, A. Liang, B. Qian, J. Fu, *Luminescence*, **25**, 431 (2010).
[42] R. Pérez-Olmos, J.C. Soto, N. Zárate, A.N. Araújo, M.C.B.S.M. Montenegro, *Anal. Chim. Acta*, **554**, 1 (2005).
[43] L.K. Shpigun, E.Yu. Andryukhina, A.S. Protasov, *J. Electroanal. Chem*., **743**, 46 (2015).
[44] L.K. Shpigun, E.Yu. Andryukhina, P.M. Kamilova, *J. Anal. Chem*., **71**, 590 (2016).
[45] P.Ü Civcir., *J. Mol. Struct. (Theochem),* **535,** 121 (2001).
[46] A. Shafaati, B.J. Clark*, Drug. Dev. Ind. Pharm*., **26,** 267 (2000).
[47] J. Rodriguez Flores, J.J. Berzas Nevado, G. Castaneda Penalvo, M.I. Rodriguez Caceres, *Chromatographia,* **57**, 493 (2003).
[48] T. Dervieux, G. Meyer, R. Barham, M. Matsutani, M. Barry, R. Boulieu, B. Nery, E. Seidman, *Clin. Chem*., **51**, 2074 (2005).
[49] W. Siangproh, W. Leesutthipornchai, W. Dungchai, O. Chailpakau, J. Flow Injection Anal., **26**, 5 (2009).
[50] W.R. Melchert, B.F. Reis, Fabio R.P. Rocha, *Anal. Chim. Acta*, **714,** 8 (2012).

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