Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

ORIGINAL ARTICLE

Luminol–K₃Fe(CN)₆ chemiluminescence system for the determination of glipizide

Xin Chen[a], Li-Li Xing[b], Yu-Hai Tang[b],*, Guang-Bin Zhang[b]

[a]*The 405 Hospital of Hanzhong City, Hanzhong, Shaanxi 723312, China*
[b]*Institute of Analytical Sciences, Xi'an Jiaotong University, Xi'an 710061, China*

Abstract A rapid and sensitive flow-injection chemiluminescence (CL) method for the determination of glipizide was developed on the basis of finding that glipizide can enhance the CL intensity of the luminol–K₃Fe(CN)₆ system. In optimum condition, the increased CL intensity was directly proportional to the concentration of glipizide in the range from 4.0×10^{-8} g/mL to 1.0×10^{-6} g/mL and the detection limit was 1.0×10^{-8} g/mL glipizide. The relative standard deviation (RSD) of the developed method was 2.1% with 11 repeated measurements of 1.0×10^{-7} g/mL glipizide. The developed method has been successfully applied to the analysis of glipizide in its pharmaceutical preparations.

1. Introduction

Glipizide (GP), 1-cyclohexyl-3-[[p-[2-(5-methylpyrazine-carboxami-do)ethyl]-phenyl]-sulfonyl]urea (as shown in Scheme 1), is a second generation sulfonylurea hypoglycemic agent and is typically used in the treatment of non-insulin dependent diabetes mellitus [1,2], which lowers the blood glucose level in humans by stimulating the release of insulin from the pancreas and helping the body to use insulin efficiently [3]. Because its effect is indeed quick and well tolerated, more importantly for a long-term use, GP has been widely used, as an ideal anti-diabetic drug with the more commonly-known name Glucotrol, in Europe as well as other regions worldwide [4]. Therefore, it is quite necessary to develop a new method for the analysis of GP.

In the past few years, several methods were reported for the determination of GP, which involved different high performance liquid chromatographies (HPLCs) [5–13], liquid chromatography–mass spectrometry (LC–MS) [14–17], ultraviolet spectrophotometry [18], thin-layer chromatography (TLC) [19] and an electrochemical method [20]. Most of these methods are tedious, inconvenient and suffer from low sensitivity and narrow linear range.
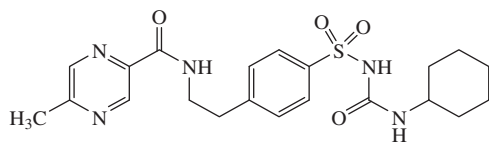
Recently, extremely sensitive analytical techniques based on chemiluminescence (CL) systems (such as NBS–H₂O₂–

*Corresponding author. Tel./fax: +86 29 82655399.
  E-mail address: tyh57@mail.xjtu.edu.cn (Y.-H. Tang).

Scheme 1 Structure of GP.

nitrofurazone, KMnO$_4$–Na$_2$SO$_3$–atenolol, luminol–potassium peroxydisulfate–bisphenol, etc. [21,22]), have been paid considerable attention, which had been applied in determining trace and ultra-trace concentrations of inorganic and organic species (like drugs or pesticides) in a variety of industrial, clinical and environmental matrices [23]. The CL method had the advantages such as high sensitivity, relatively inexpensive apparatus, short analysis time, wide working range, low detection limit and no background scattering light interference. Coupled with flow injection analysis (FIA), the reproducibility and selectivity of CL analysis can be improved significantly. However, up to now, there is no report on the use of FI-CL method for determination of GP in pharmaceutical preparations. In this work, it was found that GP could obviously enhance the CL of the luminol–K$_3$Fe(CN)$_6$ reaction in NaOH solution and the enhanced CL was proportional to the concentration of GP. So, a new flow-injection CL method was proposed and the mechanism of luminol–K$_3$Fe(CN)$_6$–GP was also discussed briefly.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared from analytical reagent grade chemicals combined with double distilled water.

A stock solution of GP (1.0×10^{-3} g/mL) was prepared by dissolving 0.1000 g of GP (National Institute of Pharmaceutical and Biological Authentication of China, Xi'an, China) in alcohol and then diluting to 100 mL.

Luminol stock solution (1.0×10^{-2} M) was obtained by dissolving 0.1771 g of luminol (Aldrich, Sigma-Aldrich Qumica) in 10 mL 0.1 M NaOH and diluting to 100 mL with water.

Potassium ferricyanide stock (1.0×10^{-2} M) was prepared by dissolving 0.1150 g K$_3$Fe(CN)$_6$ (Xi'an Chemical Reagent Factory, Xi'an, China) in distilled water and then diluting to 50 mL.

The stock solutions were stored at 4°C in a refrigerator and protected from light. The working solutions were prepared by diluting stock solutions freshly into appropriate concentration with water before being used.

2.2. Apparatus and procedures

The CL measurement was conducted on an IFFM-E flow injection CL analyzer (Xi'an Remax Electronic Science-Tech. Co. Ltd., Xi'an, China), which includes a model IFFM-E flow injection system and a model IFFS-A luminometer. A schematic representation of the proposed FI-CL system for the detection of GP is illustrated in Fig. 1. Two peristaltic pumps, labeled as P$_1$ and P$_2$, were used to deliver the respective components to the flow cell at a flow rate of 2.65 mL/min. The
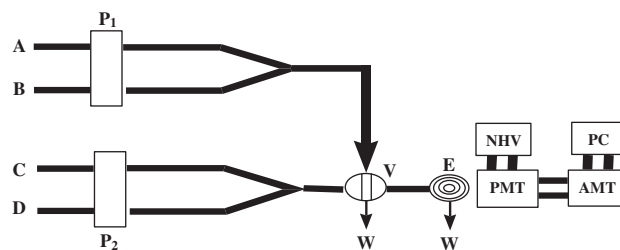

Fig. 1 Schematic diagram of the flow system for the determination of GP. A: luminol; B: NaOH; C: K$_3$[Fe(CN)$_6$]; D: sample/blank; P$_1$, P$_2$: peristaltic pumps; V: six-way valve; E: flow cell; W: waste; NHV: negative high voltage; PMT: photomultiplier; PC: computer; AMP: amplifier.
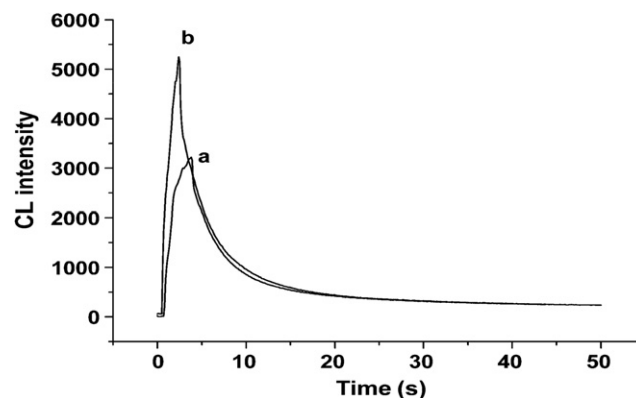

Fig. 2 Chemiluminescence kinetic curves: a, 8.0×10^{-5} M luminol+6.0×10^{-5} M K$_3$Fe(CN)$_6$; b, 8.0×10^{-5} M luminol+6.0×10^{-5} M K$_3$Fe(CN)$_6$+1.0×10^{-7} g/mL GP.

tubes A, B, C and D were used to deliver luminol solution, NaOH solution, K$_3$Fe(CN)$_6$ solution and sample or blank solution, respectively. Luminol solution was premixed with potassium ferricyanide. Then, a certain amount of GP sample was delivered into the emerging stream through the six-way injection valve. The light output from the CL reaction was detected and amplified with a photomultiplier tube and luminometer. Data acquisition and processing were performed using the IFFM-E software running under Windows XP. The determination of GP was accomplished based on the increase in the CL intensity, calculated as $\Delta I = I - I_0$, where I denotes the CL signal in the presence of GP and I_0 is the CL intensity corresponding to baseline.

3. Results and discussion

3.1. Kinetic characteristic of the CL reaction

The kinetic curves of the CL reaction of luminol–K$_3$Fe(CN)$_6$–GP system were taken on an IFFS-A multipurpose chemiluminescence detector (shown in Fig. 2). It could be seen that the oxidation of luminol with K$_3$Fe(CN)$_6$ was comparatively fast (2–3 s) and the CL intensity was relatively strong. The CL signal (3200) in the presence of GP (1.0×10^{-7} g/mL) is stronger than that (5350) in the absence of GP. The results indicated that GP could sensitize the CL emission of luminol–K$_3$Fe(CN)$_6$ system significantly.

3.2. Selection of oxidants

The characteristics of several oxidants, including $KMnO_4$, $K_3Fe(CN)_6$, KIO_4 and H_2O_2 of the same concentration reacting with luminol CL systems in the presence of GP, were evaluated. It was found that GP had more effective enhancement in luminol–$K_3Fe(CN)_6$ system, compared with other systems. Therefore, $K_3Fe(CN)_6$ was selected as an oxidant for the subsequent study.

3.3. Effect of sample injection sequences

In this work, different injection sequences were tested to optimize conditions. The strongest relative CL intensity was found when $K_3Fe(CN)_6$ solution was premixed with luminol and then with sample solution (as described in Fig. 1).

3.4. Optimization of experimental conditions

A series of experiments had been performed for optimizing the chemical and instrumental parameters affecting the analytical response, which included the component concentration, flow rate, the length of mixing tube, etc. 1.0×10^{-7} g/mL standard solution of GP was applied to optimizing the experimental conditions.

3.4.1. Effect of chemical variables
The chemical variables that need to be assessed include luminol, potassium ferricyanide and the medium. The effect of luminol concentration (the medium was 0.1 M NaOH) was investigated in the range of 8×10^{-6}–2×10^{-4} M. As shown in Fig. 3, increasing the luminol resulted in a sharp increase in the relative CL signal up to 8×10^{-5} M; after that, 1×10^{-6} g/mL glipizide could not be detected (the CL signal had reached beyond the maximum system detection) and S/N decreased although CL still increased. Considering the stability of the CL signal, a luminol concentration of 8×10^{-5} M was selected for the subsequent experiment.

The dependence of the increased CL intensity (ΔI) on the concentrations of potassium ferricyanide was examined in the range from 1.0×10^{-6} M to 1.0×10^{-3} M. The result related to 1.0×10^{-5} M–10×10^{-5} M is shown in Fig. 4. A general improvement in the CL intensity sharply increases with an increase of the concentration of potassium ferricyanide from 1.0×10^{-5} M to 4.0×10^{-5} M, and then slowly increases. 6.0×10^{-5} M potassium ferricyanide was selected to further study since a higher signal to noise ratio was obtained in this concentration solution.
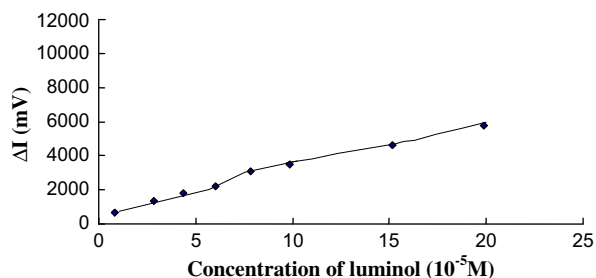

Fig. 3 Effect of luminol concentration on CL intensity: 1.0×10^{-7} g/mL glipizide; 6.0×10^{-5} M $K_3Fe(CN)_6$ and 0.1 M NaOH.
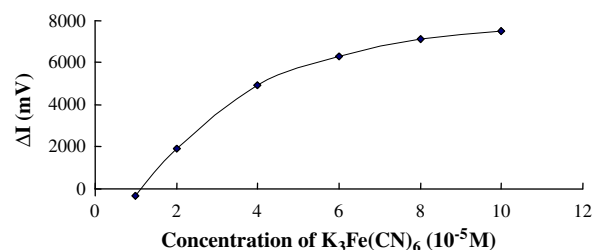
Fig. 4 Effect of $K_3Fe(CN)_6$ concentration on CL intensity: 1.0×10^{-7} g/mL glipizide and 8.0×10^{-5} M luminol (0.1 M NaOH).
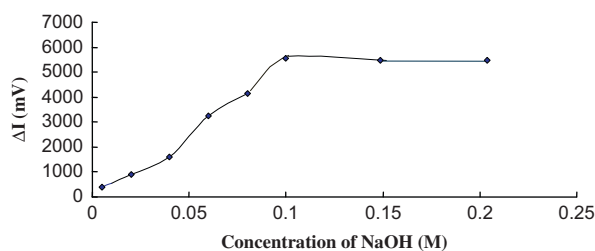
Fig. 5 Effect of NaOH concentration on CL intensity: 1.0×10^{-7} g/mL glipizide; 6.0×10^{-5} M $K_3Fe(CN)_6$; and 8.0×10^{-5} M luminol.

The CL signal was tested in several alkaline solutions, which included NaOH, Na_2CO_3, $NaHCO_3$, Na_3PO_4 and Na_2HPO_4. We observed that the CL signal was stronger when adding appropriate concentration of NaOH in the reaction system. In this work, over the range 0.005–0.2 M, the effect of the concentration of NaOH was studied and the results are shown in Fig. 5. Maximum CL response was obtained at a NaOH concentration of 0.1 M, over which the relative CL intensity signal did not increase remarkably any more. So, 0.1 M NaOH was regarded as the optimized working concentration for the subsequent work.

3.4.2. Effect of instrumental parameters
The effect of flow rate on the CL response was calibrated in terms of sensitivity, reagent consumption and speed. Flow rate V_0 was studied over the range 1.55–4.50 mL/min in each stream. The results showed that the CL response continued to increase with increasing flow rate up to 2.65 mL/min, at which maximum CL intensity was observed, and then began to drop. Thus, a flow rate of 2.65 mL/min was chosen as the suitable rate with a steady baseline and reproducible peak height.

The length of the mixing tubing (L_0) was also adjusted to yield maximum light emission in the cell. Because CL reaction is very fast, if the length of mixing tubing to the flow cell is too long or too short, the CL signal cannot be detected. In this work, the effect of L_0 was investigated over the range from 3 to 15 cm. It was found that a 10 cm mixing tubing afforded the best results with good reproducibility and sensitivity, which was selected for further work.

To sum up, the optimized conditions consisted of luminol (8×10^{-5} M) in NaOH (0.1 M) as the stream with a flow rate

of 2.65 mL/min and potassium ferricyanide (6.0×10^{-5} M) introduced to the carrier by a six-way valve selected for the purpose of optimization.

3.5. Analytical performance—calibration curve, detection limit and precision

A series of working standard solutions of GP with different concentrations (1.0×10^{-8} g/mL–1.0×10^{-4} g/mL) were prepared by diluting freshly respective stock standard solutions with water. Under the selected experimental conditions described above, ΔI, the difference in CL intensity in the absence and presence of GP, possessed a linear relationship with GP concentration ranging from 4.0×10^{-8} g/mL to 1.0×10^{-6} g/mL. The linear regression equation was expressed as $\Delta I = 19.738c + 1102.9$ ($r = 0.9901$, $n = 5$). The detection limit (3σ) of the method was estimated to be 1.0×10^{-8} g/mL and the relative standard deviation (RSD) for determination of 1.0×10^{-7} g/mL GP was 2.1% ($n = 11$), which was indicative of repeatability and reproducibility of the assay.

3.6. Interference

In order to assess the selectivity of the developed CL method, varying amounts of possible interfering substances were added to the determination of a standard GP solution (1.0×10^{-7} g/mL). The obtained signals were tested against a pure solution of GP at the same concentration. The tolerance of foreign species was taken as the largest concentration yielding a relative error less than 5% in CL signal. In all the mixed media, the determined GP concentration was in good agreement with that in the pure GP solution. The above results indicated that the common accessories found in the pharmaceutical preparations, including lactose, starch, glucose, saccharin, sucrose and magnesium stearate, had no obvious effects on the determination of GP. And the existence of 1000-fold Ca^{2+}, Mg^{2+}, K^+, SO_4^{2-}, NO_3^-; 100-fold Al^{3+}, Zn^{2+}, Ni^{2+} and 50-fold CO_3^{2-}, Cl^- did not interfere with the determination.

3.7. Stability

It was found that the response factors of standard solution were unchanged for 2 weeks at least. Thus, the solutions can be used within this period without affecting the results. Moreover, different temperatures of the CL reaction were also studied and the results showed that it had remarkable effect on CL emission signal; in this study, constant temperature of 25 °C was chosen as the suitable work temperature.

3.8. Application

The proposed FI-CL method was successfully applied to the analysis of GP commercial pharmaceutical tablets (1009141, 5 mg per tablet, Jiangsu Pingguang Pharmaceutical Group, Co., Ltd., Jiangsu, China). The sample solution was prepared by dissolving a quantity of sample equivalent to one tablet from 10 tablets and by sonication with appropriate volume of alcohol for 20 min to aid its dissolution. The dissolved sample was filtrated with Whatman No. I filter paper and diluted with distilled water to 100 mL. We diluted the sample solution to

the linear range for analysis. The result obtained (4.7 mg per tablet, $n = 5$) is in good agreement with the Chinese pharmacopoeia reference method (4.8 mg per tablet, $n = 5$) and the nominal content of GP (5.0 mg per tablet), the RSD being less than 2%. The recoveries were determined by adding 3 GP solutions (1.5×10^{-7}, 3.0×10^{-7}, 5.0×10^{-7} g/mL) respectively to the pharmaceutical preparation (2.0×10^{-7} g/mL). The determination results ($n = 9$) were 3.46×10^{-7}, 4.94×10^{-7}, 6.95×10^{-7} g/mL, correspondingly, the average recovery being 98.1%, which was in the acceptable range.
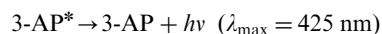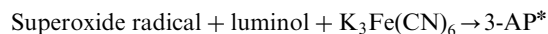
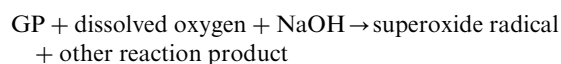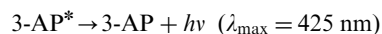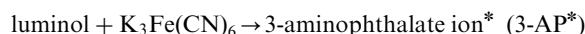### 3.9. Discussion of possible CL mechanism

In order to obtain the CL reaction mechanism, the fluorescence spectra of luminol, luminol–$K_3Fe(CN)_6$, and luminol–$K_3Fe(CN)_6$–GP reaction were scanned in the range of 280–650 nm, using an RF 5301 fluorescence spectrophotometer. It had been reported that 3-aminophthalate ion (3-AP), an oxidized product of luminol, peaking at 425 nm was known as the emitter in the luminol–$K_3Fe(CN)_6$ system [24]. The results obtained were all found to have the same maximum emission appearing at 425 nm; the luminophor was confirmed to be 3-AP. This indicated that the luminant in the luminol–$K_3Fe(CN)_6$–GP system was still 3-AP.

On the other hand, when GP standard solution, luminol solution and $K_3Fe(CN)_6$ solution were purged with nitrogen for 5 min and the dissolved oxygen was removed from all solutions, it was found that the CL intensity decreased by about 30%. The result indicated that the dissolved oxygen is required in the CL reaction.

The superoxide radical had been confirmed to oxidize luminol to produce CL in alkaline solution [25]. The increasing agents could increase dissolved oxygen to intermediate superoxide radical in alkaline solution. The produced superoxide radical reacts with luminol to yield an unstable endoperoxide leading to an excited aminophalate. The CL reaction of luminol with superoxide radical can be catalyzed by potassium ferricyanide in alkaline solution.

Based on the discussion described above, the possible CL reaction mechanism was considered as follows:

$$\text{luminol} + K_3Fe(CN)_6 \rightarrow \text{3-aminophthalate ion}^* \ (\text{3-AP}^*)$$

$$\text{3-AP}^* \rightarrow \text{3-AP} + hv \ (\lambda_{max} = 425 \text{ nm})$$

$$\text{GP} + \text{dissolved oxygen} + \text{NaOH} \rightarrow \text{superoxide radical} + \text{other reaction product}$$

$$\text{Superoxide radical} + \text{luminol} + K_3Fe(CN)_6 \rightarrow \text{3-AP}^*$$

$$\text{3-AP}^* \rightarrow \text{3-AP} + hv \ (\lambda_{max} = 425 \text{ nm})$$

4. Conclusion

A sensitive FI-CL method for the determination of antidiabetic drug—GP based on luminol and potassium ferricyanide in NaOH was proposed for the first time in the present work. It had been proved that this method is sensitive, simple and rapid to analyze GP in pharmaceutical preparation based on its sensitizing effect. The result obtained was in reasonable agreement with that achievable by the Chinese pharmacopoeia

method. If combined with separation techniques, the system may be adopted to detect GP in other samples.

Acknowledgments

References

[1] S. Mutalik, N. Udupa, Pharmacological evaluation of membrane-moderated transdermal system of glipizide, Clin. Exp. Pharmacol. 33 (1–2) (2006) 17–26.

[2] K.D. Tripathi, Insulin, oral hypoglycaemics and glucagons, Essentials of Medical Pharmacology, fifth ed., Jaypee Brothers Publishing House, New Delhi, 2004, p. 235.

[3] Z.J. Lin, D. Desai-Krieger, L. Shum, Stimultaneous determination of glipizide and rosiglitazone unbound drug concentrations in plasma by equilibrium dialysis and liquid chromatography–tandem mass spectrometry, J. Chromatogr. B—Anal. Technol. Biomed. Life Sci. 801 (2) (2004) 265–272.

[4] ⟨http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a684060.html⟩, 2011.

[5] A. Dubey, I.C. Shukla, Simultaneous determination of glipizide and metformin hydrochloride in pharmaceutical preparation by HPLC, J. Indian Chem. Soc. 81 (1) (2004) 84–86.

[6] K.S. Lakshmi, T. Rajesh, Development and validation of RP-HPLC method for simultaneous determination of glipizide, rosiglitazone, pioglitazone, glibenclamide and glimepiride in pharmaceutical dosage forms and human plasma, J. Iran. Chem. Soc. 8 (1) (2011) 31–37.

[7] S. Gupta, G. Bansal, Validated stability-indicating HPLC-UV method for simultaneous determination of glipizide and four impurities, J. AOAC Int. 94 (2) (2011) 523–530.

[8] B. Jung-Woo, K. Nam-Tae, C. Chang-Ik, et al., HPLC analysis of plasma glipizide and its application to pharmacokinetic study, J. Liq. Chromatogr. Relat. Technol. 32 (13) (2009) 1969–1977.

[9] L. Tang, J. Huang, Z.H. Guo, Determination of metformin, glipizide and cinnamaldehyde in the compound preparation by HPLC, Chin. J. Pharm. 39 (5) (2008) 371–372.

[10] J.L. Tang, L. Wang, R.F. Xiang, et al., Determination of glipizide in human serum by HPLC, Chin. J. Anal. Lab. 26 (2) (2007) 84–88.

[11] S. Vijaya Saradhi, V. Satya Kiran, V. Hima Bindu, et al., High pressure liquid chromatography estimation of glipizide in pharmaceutical dosage forms, Asian J. Chem. 18 (2) (2006) 1309–1312.

[12] X.O. Wu, L.H. Xiao, Y. Xing, Determination of glipizide mixed illegally in traditional Chinese medicine preparations by HPLC, J. Chin. Pharm. 09 (1) (2006) 46–47.

[13] S. AbuRuz, J. Millership, J. McElnay, The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma, J. Chromatogr. B—Anal. Technol. Biomed. Life Sci. 817 (2) (2005) 277–286.

[14] C.G. Ding, Z. Zhou, Q.H. Ge, et al., Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography–tandem mass spectrometry, J. Biomed. Chromatogr. 21 (2) (2007) 132–138.

[15] F.Y. Liu, Y.C. Xie, F.Q. Liu, et al., LC–MS determination of metformin, phenformin, glipizide and glyburide illegally mixed into two kind of antidiabetic preparations of traditional Chinese medicine, Chin. J. Pharm. Anal. 29 (3) (2009) 440–444.

[16] G. Bansal, M. Singh, K.C. Jindal, et al., LC and LC–MS study on establishment of degradation pathway of glipizide under forced decomposition conditions, J. Chromatogr. Sci. 46 (6) (2008) 510–517.

[17] X.H. Zhao, B. Song, D.F. Zhong, et al., Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography–tandem mass spectrometry, Acta Pharm. Sin. 42 (10) (2007) 1087–1091.

[18] B.H. Chen, X.R. Zhang, UV spectrophotometry of glipizide tablets, Chin. J. Pharm. 23 (3) (1992) 124–125.

[19] A. Gumieniczek, A. Berecka, Quantitative analysis of gliclazide and glipizide in tablets by a new validated and stability-indicating RPTLC method, J. Planar Chromatogr. Mod. TLC 23 (2) (2010) 129–133.

[20] A. Radi, T. Wahdan, N. Abd El-Ghany, Electrochemical oxidation of the hypoglycaemic drug glipizide on carbon paste electrode and its determination in tablet dosage form, Chem. Anal. 49 (5) (2004) 627–633.

[21] P. Thongsrisomboon, B. Liawruangrath, S. Liawruangrath, et al., Determination of nitrofurans residues in animal feeds by flre injection chemiluminescence procedure, Food Chem. 123 (3) (2010) 834–839.

[22] Y.F. Zhao, N. Zhang, Q. Wei, et al., Studies and application of flow injection chemiluminescence in analytical chemistry, Spectrosc. Spectral Anal. 30 (9) (2010) 2512–2517.

[23] B.C. Xue, T. Wang, E.B. Liu, Recent progress in flow-injection chemiluminescence for pharmaceutical analysis, Spectrosc. Spectral Anal. 26 (5) (2006) 816–820.

[24] H.Y. Liu, L. Zhang, J.M. Zhou, et al., Flow injection chemiluminescence determination of dobutamine hydrochloride injection using luminol–ferricyanide/ferrocyanide system, Anal. Chim. Acta 541 (1–2) (2005) 123–127.

[25] J. Du, Y. Li, J. Li, Flow injection chemiluminescence determination of captopril based on its enhancing effect on the luminol–ferricyanide/ferrocyanide reaction, Luminescence 17 (3) (2002) 165–167.