Anal Bioanal Chem (2004) 380: 918–923
DOI 10.1007/s00216-004-2841-5

ORIGINAL PAPER

Yao-Dong Liang · Jun-Feng Song · Tian Tian

Determination of pipemidic acid based on flow-injection chemiluminescence due to energy transfer from peroxynitrous acid synthesized on-line

Received: 26 May 2004 / Revised: 20 August 2004 / Accepted: 26 August 2004 / Published online: 26 November 2004

Abstract A flow-injection chemiluminescence (CL) method for the determination of pipemidic acid is described. It is based on energy transfer from excited state peroxynitrous acid to pipemidic acid, in which the excited state peroxynitrous acid is synthesized on-line by the mixing of acid hydrogen peroxide with nitrite in a flow system and the CL is from two excited states of pipemidic acid. The proposed method allows the measurement of pipemidic acid over the range of 2.0×10^{-7}–2.0×10^{-5} mol l^{-1} . The detection limit is 6.3×10^{-8} mol l^{-1}, and the relative standard deviation for 2.0×10^{-6} mol l^{-1} pipemidic acid ($n = 9$) is 0.9%. This method was evaluated by the analysis of pipemidic acid in pharmaceutical preparations.

Keywords Pipemidic acid · Peroxynitrous acid · Flow injection · Chemiluminescence (CL)

Introduction

Pipemidic acid (Scheme 1) is an antibacterial agent used to treat gram-negative urinary tract infections [1], but it also severely damages DNA in the absence of an exogenous metabolizing system [2]. Several analytical techniques have been reported for the determination of pipemidic acid, including titrimetry [3], spectrophotometry [4, 5], fluorimetry [6, 7], HPLC [8], capillary electrophoresis [9, 10], electrochemical [11, 12] and chemiluminescence (CL) methods [13–16]. Among the CL methods, one is based on the oxidation reaction between pipemidic acid and on-line electrogenerated cobalt(III) [13]. Another is a sulfite-based CL method, based on the sensitizing effect of pipemidic acid on the weak CL reaction between sulfite and permanganate or bismuthate [14, 15], or based on the sensitizing effect of the pipemidic acid-terbium(III) complex on the weak CL reaction between sulfite and cerium(IV) [16]. However, the use of a cobalt(III) or solid bismuthate system requires a comparatively complicated CL set-up [17], the cerium(IV) system consumes an expensive reagent (terbium(III)), and the permanganate system suffers from an overlap between the CL band and the absorption band of permanganate [18]. A simple and convenient method that can be used to measure pipemidic acid with high sensitivity is not yet to be reported.

Peroxynitrous acids (ONOOH), including *cis*-ONOOH, *trans*-ONOOH and excited state ONOOH ($ONOOH^*$), possess strong oxidizing and peroxidizing abilities, and $ONOOH^*$ is also potent [19–23]. Peroxynitrous acids used as unstable reagents are conventionally synthesized on-line by the mixing of acid hydrogen peroxide with nitrite in a flow system [24]. Therefore, peroxynitrous acids have potential applications in CL analysis. However, to the best of our knowledge, reports on their application in CL analysis are scarce [25–27].

In this work, we discuss the observation that intense CL emission occurs when pipemidic acid is present in the acidic hydrogen peroxide–nitrite CL system. The mechanism of this intense CL is discussed in detail. A rapid and sensitive FI-CL method for the determination of pipemidic acid is then proposed, and this proposed method is evaluated by analyzing pipemidic acid in pharmaceutical preparations.

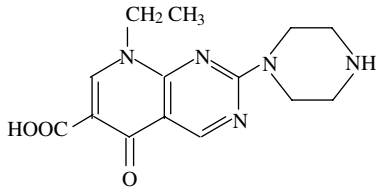
Y.-D. Liang · J.-F. Song (✉) · T. Tian
Institute of Analytical Science, Northwest University,
Xi'an 710069, P.R. China
E-mail: songjunf@nwu.edu.cn

Y.-D. Liang
Department of Chemistry and Chemical Engineering,
Xi'an University of Science and Technology,
Xi'an 710054, P.R. China

Experimental

Reagents

Pipemidic acid was of biochemical-reagent grade and was purchased from the National Institute for the Control of Pharmaceutical and Biological Products



Scheme 1 Structural formula of pipemidic acid

(Beijing, China). A stock standard solution of pipemidic acid (1.00×10^{-3} mol l^{-1}) was prepared by dissolving 0.0357 g of pipemidic acid in 0.01 mol l^{-1} sodium hydroxide solution, and diluting to 100 ml with the same sodium hydroxide solution. The standard working solutions were prepared by diluting the stock solution with water before they were used. Acidic hydrogen peroxide solution (0.24 mol l^{-1}) was prepared by diluting 30% (v/v) hydrogen peroxide (Xi'an Chemical Reagent Plant, Xi'an, China) with 0.04 mol l^{-1} sulfuric acid solution and was standardized with standard potassium permanganate. Other chemicals used were of analytical reagent grade. Double-distilled water was used throughout the experiments.

Apparatus

The flow injection manifold is shown in Fig. 1. An IFFM-D CL analyzer (Xi'an Remax Electronic Science-Tech Co. Ltd., China) was employed to deliver the solutions and to measure CL emission. It consisted of two peristaltic pumps, a six-way injection valve, a Y-shaped mixing element (Y), a flow cell and a photomultiplier tube (PMT). All components were connected with PTFE tubes (0.8 mm i.d.). A mixing coil (glass tube, 100×1 mm i.d.) was used as flow cell and was placed in front of the PMT (Model R105UH, Hamamatsu, Japan). Injection was made using a six-way injection valve equipped with a 100 µl sample loop. The CL signal was collected with the PMT and recorded with a computer equipped with CL analysis system software (Xi'an Remax Electronic Science-Tech Co. Ltd., China).

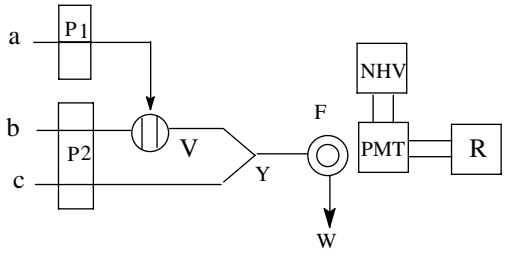


Fig. 1 Schematic diagram of the flow injection CL manifold used for the determination of pipemidic acid: **a** sample solution, **b** 0.20 mol l^{-1} sodium nitrite solution, **c** 0.24 mol l^{-1} hydrogen peroxide-0.04 mol l^{-1} sulfuric acid solution; *P*1 and *P*2 peristaltic pumps, *Y* Y-shaped mixing element, *V* six-way valve, *F* flow cell, *W* waste, *NHV* negative high voltage, *PMT* photomultiplier tube, *R* computer

The PMT was operated at −900 V. The fluorescence spectra were monitored using a RF-540 fluorescence spectrometer (Shimadzu, Japan.).

Calibration procedure

By keeping the valve in the washing position, acidic hydrogen peroxide and nitrite solutions were continuously pumped into the manifold until the baseline was established on the recorder. Then, 100 µl of the standard working solutions or sample solution was injected into the nitrite solution. The nitrite solution was merged with a stream of acidic hydrogen peroxide in the Y-shaped mixing element (Y) and this then reached the flow cell, producing CL emission. The CL signal produced in the flow cell was recorded. A calibration graph was constructed by plotting the CL intensity of the CL signal vs. the concentration of pipemidic acid in the standard working solutions.

Procedure for pipemidic acid

Pipemidic acid tablets (Xi'an Pharmaceutical Plant, China), containing pipemidic acid, starch, dextrin and glucose, were purchased from a local hospital. Ten tablets of pipemidic acid were weighed and pulverized. An accurately weighed amount of the powder equivalent to about 35.7 mg (0.1 mmol) of pipemidic acid was dissolved in 0.01 mol l^{-1} sodium hydroxide and diluted to 100 ml with the sodium hydroxide solution. After filtering, aliquots of the filtrate were further diluted with water so that the final pipemidic acid concentration was within the working range. The determination was performed according the procedures mentioned above.

Procedure for CL spectrum and CL kinetic profiles

The CL spectrum was obtained via a set of interference filters. The filters were set between the flow cell and PMT. The flow injection method described above was used to obtain the CL emission at different wavelength bands.

CL kinetic profiles were obtained using a batch method. 5.0 ml acidic hydrogen peroxide was pipetted into a reaction cell in front of the PMT. 5.0 ml of pipemidic acid solution, sodium nitrite-pipemidic acid mixed solution or sodium nitrite solution was rapidly injected into the reaction cell through a fill orifice using an injector. The CL signal produced was recorded by the flow injection CL analyzer.

Results and discussion

Characteristics of chemiluminescence

The experiments showed that mixing acidic hydrogen peroxide with nitrite produced a weak CL (Fig. 2b).

This CL originated from excited state peroxynitrous acid ($ONOOH^*$) [28]. When pipemidic acid was added to the weak CL system, the CL intensity increased greatly (Fig. 2c).

In order to demonstrate the mechanism for the intense CL, a series of experiments were performed. First, mixing pipemidic acid solution with a strong oxidant such as cerium(IV), permanganate and periodate instead of peroxynitrous acid in sulfuric acid did not produce any CL. Moreover, no CL was observed when pipemidic acid was mixed solely with either hydrogen peroxide or nitrite in sulfuric acid medium. These results indicated that the intense CL did not result from oxidation of the pipemidic acid by the on-line synthesized peroxynitrous acid.

Second, the fluorescence and CL spectra were examined. When the fluorescence spectrum was recorded in the range of 300–700 nm in pipemidic acid-sulfuric acid solution, using an excitation wavelength at $\lambda_{max,\ ex} =$ 380 nm, only one fluorescence peak ($\lambda_{max,\ em} = 450$ nm) was observed. The fluorescence peak was from pipemidic acid in the ketone form (I) [29]. After hydrogen peroxide or sodium nitrite was added separately into the pipemidic acid–sulfuric acid solution, the fluorescence spectrum and the peak intensity ($\lambda_{max,\ em} = 450$ nm) hardly changed. Moreover, after adding a small amount of sodium nitrite into pipemidic acid–hydrogen peroxide–sulfuric acid solution, the wavelength of the fluorescence peak did not change while the peak intensity ($\lambda_{max,\ em} = 450$ nm) decreased to a certain degree. This decrease in the peak intensity indicated that the intense CL was due to energy transfer from $ONOOH^*$ to pipemidic acid in the ketone form and it indicated that the excited state pipemidic acid would isomerize to some other form, such as the enol, in acid solution [29]. Moreover, CL spectra from acidic hydrogen peroxide–nitrite solution in the absence and the presence of pipemidic acid were also recorded in the range 400–680 nm using the flow-injection method. When pipemidic acid was absent, no obvious CL spectrum was observed (Fig. 3a). The reason was that the CL from $ONOOH^*$ was too weak to penetrate the interference filters. The CL spectrum in the presence of pipemidic acid showed two peak bands, 420–490 and 490–575 nm (Fig. 3b). The first band (420–490 nm) coincided with the fluorescence spectrum of pipemidic acid in the ketone form (I) ($\lambda_{max,\ em} = 450$ nm), obtained in this work via pipemidic acid–sulfuric acid solution. The second band (490–575 nm) correlated with the CL spectrum of pipemidic acid in the enol form (II) ($\lambda_{max,\ em} = 526$ nm), as the isomerization product of pipemidic acid in the ketone form [29, 30].

Additionally, previous work has proved that pipemidic acid can easily accept energy transfers and it has high fluorescence quantum efficiency in acid solution [29]. When pipemidic acid was present in the sulfite-based CL system, the energy from the excited state sulfide dioxide was transferred to the pipemidic acid, which was responsible for the intense CL [14, 15]. The weak CL resulted from $ONOOH^*$. The intense CL from pipemidic acid in the present work should be attributed to energy being transferred from $ONOOH^*$ to pipemidic acid in the ketone form (I), forming excited state pipemidic acid in the ketone form (I^*). Then, some of the excited state pipemidic acid in ketone form isomerized further to excited state pipemidic acid in enol form (II^*). The intense CL was the result of both excited state pipemidic acids, I^* and II^*, falling back to their ground states. The intense CL mechanism demonstrated in the present work can therefore be summarized as follows:

Optimizing the experimental conditions

A series of experiments were conducted to establish the optimum analytical conditions for the determination of

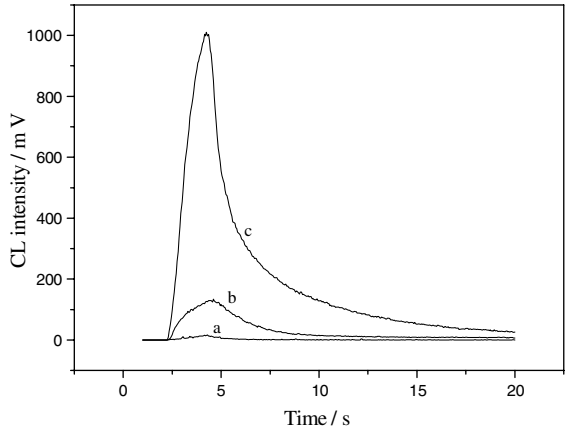


Fig. 2 CL dynamic response curves for the CL reaction of 0.24 mol l^{-1} hydrogen peroxide in 0.04 mol l^{-1} sulfuric acid solution with **a** 2.0×10^{-6} mol l^{-1} pipemidic acid, **b** 0.20 mol l^{-1} sodium nitrite, and **c** 0.20 mol l^{-1} sodium nitrite and 2.0×10^{-6} mol l^{-1} pipemidic acid in batch mode

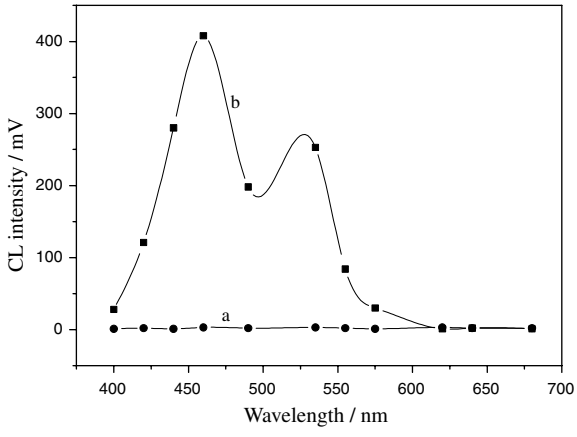
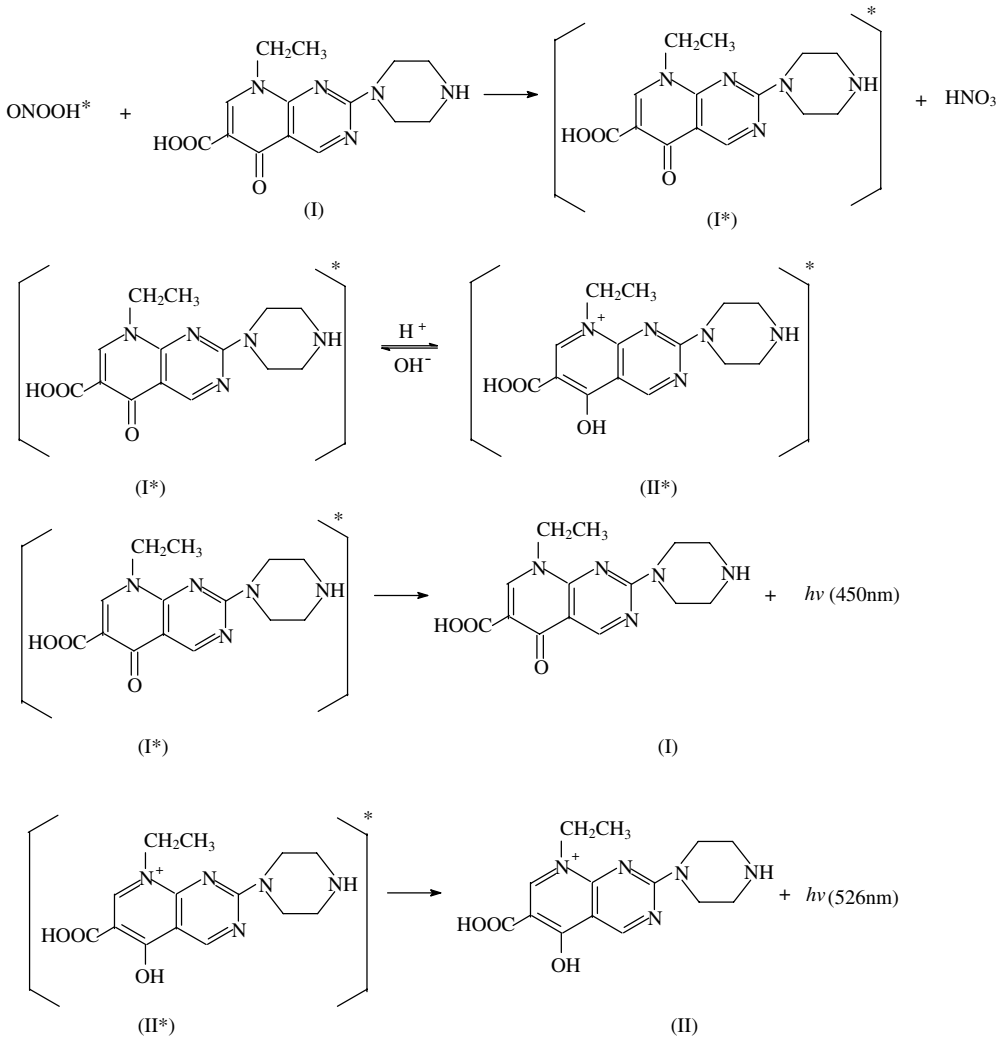


Fig. 3 CL spectra of the pipemidic acid-sensitized CL reaction of hydrogen peroxide and sodium nitrite (**b**) and the non-sensitized CL reaction (**a**). Hydrogen peroxide, 0.24 mol l^{-1}; sulfuric acid, 0.04 mol l^{-1}; sodium nitrite, 0.20 mol l^{-1}; pipemidic acid, 6.0×10^{-6} mol l^{-1}

Scheme 2



pipemidic acid. The parameters optimized included the hydrogen peroxide medium, the hydrogen peroxide and nitrite concentrations, as well as the flow rate for the FIA system.

Selecting the acid medium in the hydrogen peroxide solution

Because peroxynitrous acids do not form in alkaline or neutral media [24], no CL was observed when nitrite–pipemidic acid solution was mixed with hydrogen peroxide in alkaline or neutral media. The intense CL from the pipemidic acid–nitrite–hydrogen peroxide solution was observed when inorganic acids such as HCl, H_2SO_4, HNO_3, H_3PO_4 and $HClO_4$ were used as the hydrogen peroxide medium. When sulfuric acid was used, the intense CL signal reached its maximum value, and the best reproducibility for monitoring pipemidic acid was obtained. Thus, sulfuric acid was selected for determining pipemidic acid in the proposed CL system.

The effect of sulfuric acid concentration on the CL intensity was examined in the range 0–0.30 mol l^{-1} sulfuric acid. The maximum CL intensity was obtained when the sulfuric acid concentration was 0.04 mol l^{-1}. Therefore, 0.04 mol l^{-1} sulfuric acid was chosen for the experiments.

Effect of sodium nitrite concentration

The effect of sodium nitrite concentration on the CL intensity was investigated in the range 0.04–0.40 mol l^{-1} sodium nitrite. The CL intensity rose with as the concentration of sodium nitrite increased from 0.04–0.20 mol l^{-1}, and reached its maximum value at 0.20 mol l^{-1}. When the nitrite concentration topped 0.20 mol l^{-1} it caused the CL intensity to decrease. This decrease results from the fast reaction of nitrite with the hydroxyl radical · OH (rate constant $k = 1 \times 10^{10}$ mol^{-1} l s^{-1}) that is the decomposition product of *trans*-ONOOH [19, 31]. Therefore, 0.20 mol l^{-1} sodium nitrite was chosen for the experiments.

Effect of hydrogen peroxide concentration

The effect of hydrogen peroxide concentration on the CL intensity was investigated over the range 0.04–0.40 mol l^{-1}. The CL intensity increased as the concentration of hydrogen peroxide was increased from 0.04 to 0.24 mol l^{-1}, and reached a maximum value at 0.24 mol l^{-1} hydrogen peroxide. Therefore, 0.24 mol l^{-1} hydrogen peroxide was chosen for the experiments.

Effect of flow rate

Pump P2 was used to deliver the nitrite and acidic hydrogen peroxide solutions. As the distance between Y and the flow cell was increased, the amount of peroxynitrous acid reaching the flow cell dropped because peroxynitrous acid is a short-lived species ($t_{1/2} \sim 1$ s) [32, 33]. The distance between Y and the flow cell was therefore made to be as short as possible, and the flow rate of pump P2 was increased in order to maximize the amount of peroxynitrous acid reaching the flow cell. The effect of the flow rate of pump P2 on the CL intensity was examined in the range 1.4–6.3 ml min^{-1}. The CL signal increased sharply with increasing flow rate in the range 1.4–3.4 ml min^{-1}. When the flow rate was higher than 3.4 ml min^{-1} the CL signal reached its maximum value, and remained almost constant in the range 3.4–4.9 ml min^{-1}. When the flow rate was higher than 4.9 ml min^{-1}, the precision of the CL signal dropped significantly. It may be that a higher flow rate results in irreproducible mixing of solutions at the Y-shaped mixing element (Y) [34]. Thus a flow rate of 4.2 ml min^{-1} was chosen for the experiments.

Interference studies

The effects of common excipients used in pharmaceutical preparations, co-existing ions and other compounds were studied by analyzing synthetic sample solutions containing 2.0×10^{-6} mol l^{-1} pipemidic acid and various amounts of each interfering species. The tolerable limit for a foreign species was taken as the largest amount yielding a relative error of less than 5% for the determination of pipemidic acid. The results from the interference study are shown in Table 1. The results show that the proposed method has good selectivity; only ten-fold amounts of Cu^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+} caused negative interference. This negative interference resulted from the reaction of nitrite with hydroxyl radicals that are produced from the reaction of hydrogen peroxide with the ions mentioned above in acid solution [35].

Analytical characteristics

Under the experimental conditions selected, the linear range was 2.0×10^{-7} to 2.0×10^{-5} mol l^{-1}, and the limit of detection ($s/n = 3$) was 6.3×10^{-8} mol l^{-1}. The regression equation was $I = 10.2 + 6.8\times10^{7}C$ (where I is CL intensity and C is pipemidic acid concentration; units are millivolts and mols per liter, respectively) with a correlation coefficient of 0.9998 ($n = 11$). The precision of the proposed method was good, as indicated by a R.S.D. of 0.9% for nine replicate determinations of 2.0×10^{-6} mol l^{-1} pipemidic acid standard solution. The sample measurement frequency was calculated to be about 50 samples h^{-1}.

Table 1 Tolerable concentration ratios of some interfering species with respect to 2.0×10^{-6} mol l^{-1} pipemidic acid

Substance	Tolerable concentration ratio
Cations	
Zn^{2+}, Pb^{2+}, Mg^{2+}, K^{+}, Na^{+}, Ca^{2+}, NH_4^{+}	1,000
Mn^{2+}, Al^{3+}, Cd^{2+}, Ag^{+}, Ni^{2+}	100
Cu^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}	10
Anions	
Cl^{-}, SO_4^{2-}, PO_4^{3-}, NO_3^{-}, Ac^{-}	1,000
Vitamins	
Thiamine hydrochloride (vitamin B_1)	1,000
Ascorbic acid (vitamin C)	100
Folic acid (vitamin Bc), riboflavin (vitamin B_2)	50
Amino acid	
L-valine, L-serine, L-arginine,	1,000
L-threonine, L-cystine, L-glutamic acid	500
L-histidine, L-lysine, L-tyrosine	100
Others	
Oxalic acid, starch, urea, uric acid, dextrin	1,000
Glucose, sucrose	100
Polyethylene glycol 6000, sodium lauryl sulfate	50

Applications

The proposed method was applied to the determination of pipemidic acid in tablets. The results, shown in Table 2, agreed well with those obtained by the UV method (Pharmacopoeia method) [36]. Recovery studies were also carried out on each sample solution, to which known amounts of pipemidic acid standard solution were added. Each recovery was calculated by comparing the results obtained before and after the addition. As shown in Table 2, the recoveries were between 98 and 104%.

Conclusion

A flow-injection CL method has been proposed for the determination of pipemidic acid based on energy transfer from excited state peroxynitrous acid ($ONOOH^{*}$) to pipemidic acid. The proposed method is simpler and more sensitive than those using cobalt(III) [13], permanganate and bismuthate [14, 15], and more convenient and cheaper than that using cerium(IV) [16]. It could be applied to determinations of and pharmacokinetic research into pipemidic acid in biological samples if it was used as the detector in CE or HPLC. In addi-

Table 2 Determination of pipemidic acid in tablets

Sample	Label value (mg)	Amount (mg)		Added ($\times 10^{-6}$ mol l^{-1})	Found[c] ($\times 10^{-6}$ mol l^{-1})	Recovery (%)
		Proposed method[a]	Official method[b]			
1	250	256 ± 3	254 ± 2	0.80	0.83 ± 0.02	104
				4.00	4.03 ± 0.04	101
				8.00	8.16 ± 0.11	102
2	250	249 ± 2	248 ± 2	0.80	0.79 ± 0.02	99
				4.00	4.07 ± 0.03	102
				8.00	7.81 ± 0.14	98

[a,b,c]Mean value ± S.D. ($n = 5$)

tion, peroxynitrous acids with strong oxidizing and peroxidizing abilities can be used to determine quinolines and isoquinolines too.

Acknowledgements We would like to thank the Nature Science Foundation of Shaanxi Province (Grant No. 2002B01) for their financial support of this work.

References

1. Naber KG (2000) J Antimicrob Chemother 46:49–52
2. Mersch-Sundermann V, Hauff KH, Braun P, Lu W (1994) Int J Oncol 5:855–859
3. Wei WL, Li YW (1999) Chin J Anal Chem 27:1405–1407
4. Fuster Mestre Y, Lahuerta Zamara L, Martimez Calataynd J (2001) Anal Chim Acta 438:93–102
5. Xuan CS, Wang ZY, Song JL (1998) Anal Lett 31:1185–1195
6. Egorova A, Beltyukova S, Teslyuk O (1999) J Pharm Biomed Anal 21:585–590
7. Liu C, Zhao HC, Jin LP (1999) Spectrosc Spec Anal 19:447–449
8. Durán Merás I, Galeano Díaz T, Rodríguez Cáceres MI, Salinas López F (1997) J Chromatogr A 787:119–127
9. Hernández M, Borrull F, Calull M (2000) J Chromatogr B 742:255–265
10. Fierens C, Hillaert S, Van Den Bossche W (2000) J Pharm Biomed Anal 22:763–772
11. Corti P, Corbini G, Gratteri P, Furlanetto S, Pinzanti S (1994) Int J Pharm 111:83–87
12. He YN, Chen HY (1997) Electroanalysis 9:1426–1428
13. Li B, Zhang Z, Mu M (2000) Microchim Acta 134:223–227
14. Li LQ, Wu YY, Feng ML, Lu JR (2002) Chin J Anal Chem 30:169–171
15. Li BX, Zhang ZJ, Zhao LX, Xu CL (2002) Anal Chim Acta 459:19–24
16. Chen SL, Zhao HC, Sun CY, Lian N, Jin LP (2002) Anal Lett 35:1705–1714
17. Lau C, Lu JZ, Kai M (2004) Anal Chim Acta 503:235–239
18. Huang YM, Zhang C, Zhang XR, Zhang ZJ (1999) Anal Chim Acta 391:95–100
19. Goldstein S, Czapski G (1995) Inorg Chem 34:4041–4048
20. Houk KN, Condroski KR, Pryor WA (1996) J Am Chem Soc 118:13002–13006
21. Goldstein S, Meyerstein D, Eldik RV, Czapski G (1997) J Phys Chem A 101:7114–7118
22. Murphy MP, Packer MA, Scarlett JL, Martin SW (1998) Gen Pharmacol 31:179–186
23. Coddington JW, Hurst JK, Lymar SV (1999) J Am Chem Soc 121:2438–2443
24. Saha A, Goldstein S, Cabelli D, Czapski G (1998) Free Radical Biol Med 24:653–659
25. Liang YD, Song JF, Yang XF, Gou W (2004) Talanta 62:757–763
26. Liang YD, Song JF, Yang XF (2004) Anal Chim Acta 510:21–28
27. Lu C, Lin JM, Huie CW, Yamada M (2004) Anal Chim Acta 510:29–34
28. Starodubtseva MN, Cherenkevich SN, Semenkova GN (1999) J Appl Spectrosc 66:473–476
29. Du LM, Ji WJ, Dong C, Liu CS (2001) Spectrosc Spec Anal 21:518–520
30. Papadopoulos K, Triantis T, Dimotikali D, Nikokavouras J (2000) Anal Chim Acta 423:239–245
31. Goldstein S, Squadrito GL, Pryor WA, Czapski G (1996) Free Radical Biol Med 21:965–974
32. Kikuchi K, Nagano T, Hayakawa H, Hirata Y, Hirobe M (1993) Anal Chem 65:1794–1799
33. Mikuška P, Večeřa Z, Zdráhal Z (1995) Anal Chim Acta 316:261–268
34. Růžička J, Hansen EH (1980) Anal Chim Acta 114:19–44
35. Cheng FC, Jen JF, Tsai TH (2002) J Chromatogr B 781:481–496
36. Editorial Committee of China Pharmacopoeia (2000) China Pharmacopoeia, Part II. China Chemical Industry Press, Beijing, p 289