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A Rapid Flow Injection Spectrophotometric Analysis for Tetracycline Chlortetracycline or Oxytetracycline

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ABSTRACT

A rapid flow injection (FI) spectrophotometric procedure for the determination of tetracycline(TC), chlortetracycline(CTC) or oxytetracycline(OTC) has been developed. The method is based on the injection of 150 µL standard or sample solution into a reagent stream of iron (III) (500 µg/mL in 5.0×10^{-3} mol/L HNO_3) with the optimum flow rate of 4.0 mL/min. The brown iron (III)-tetracycline complex is monitored at 423 nm. The flow injection system and the experimental conditions were optimized by means of univariate method. Under the optimum conditions, calibration graphs were obtained for 1.0 - 100.0 µg/mL and the detection limits (s/n = 3) were 0.1 µg/mL of each drug. The correlation coefficients were 0.995, 0.994 and 0.990 for tetracycline, chlortetracycline and oxytetracycline, respectively. The method was successfully applied to the determination of these drugs in pharmaceutical formulations with a sample throughput of 70/h.

Key words: flow injection, tetracycline, chlortetracycline, oxytetracycline

INTRODUCTION

Tetracycline antibiotics are potent, broad spectrum antibacterial agents effective against a host of Gram positive and Gram negative aerobic and anaerobic bacteria. As a result, the tetracyclines are drugs of choice, or well-accepted alternatives for a variety of infectious diseases[1]. Since the first member of the tetracycline family, chlortetracycline (CTC) was discovered in 1948 and eight TCs have become commercially available. Of these TCs, tetracycline(TC), oxytetracycline (OTC) and chlortetracycline (CTC) are commonly applied to food-producing animals (including honeybees) as veterinary drugs and feed additives because of their broad spectrum activity and cost effectiveness[2]. Various methods have been developed for their determination both in pharmaceutical preparations and biological samples including UV-visible spectrophotometry[3-5], fluorimetry[6-9], electrochemical method[10-13], an immunoaffinity-based procedure[14], liquid chromatography[15-18], capillary electrophoresis[19] and chemiluminescence[20-23].

A method based on the flow injection (FI) is a well-known technique that offers improvement in most batch methods, providing high sample throughput rate, simple sample preparation and instrumentation[24]. The spectrophotometric FI method detections for tetracyclines and its derivatives are based on their abilities to bind with metals such as iron[4,5], copper[25] and aluminium[26]. However, these methods have limited concentration range and detection.

This paper examines a rapid, sensitive and reproducible flow injection method for determination of tetracycline, chlortetracycline or oxytetracycline based on the spectrophotometric detection of the soluble brown complex formed by the reaction between each drug and iron (III) in acidic solution. Result of this complex is monitored at 423 nm. Under the optimized conditions, these drugs were determined within the reasonable concentration range and the optimum limit of detection. The proposed method has significantly improved both working range and limit of detection.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

All chemicals were of analytical reagent grade and were used without further purifications. All solutions were prepared with distilled deionized water.

The hydrochlorides of tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC) were purchased from Sigma (Poole, Dorset, UK). Stock standard solutions (100 µg/mL) were prepared by dissolving 0.1000 g of each drug in water and diluting to 1000 mL with water. The iron (III) solution (500 µg/mL) was obtained

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by dissolving 4.3 g of $NH_4Fe(SO_4)_2.12H_2O$ (Fluka, Gillingham, Dorset, UK) and diluting to 1000 mL in a volumetric flask with 5.0×10^{-3} mol/L nitric acid.

II. *Apparatus*

The flow injection manifold consisted of a peristaltic pump (Eyela® MP3A, Tokyo Rikakikai Co. Ltd., Japan), and the sample or standard solution was injected via a four-way PTFE rotary valve with a 150 µL sample loop (Rheodyne® model 5041, Cotati, CA). PVC tubing (Elkay, Galway, Ireland) with 0.8 mm i.d. was used as a flow line for an ammonium iron (III) sulfate reagent, followed by injection of the sample or standard into an ammonium iron (III) sulfate in acidic solution. A mixing coil used was made from PTFE tubing, 0.8 mm i.d. and 75 cm in length for the recommended configuration. The FI peaks were acquired by using a UV-visible detector (Thermo Separation Product, TSP UV-2000, USA), coupled with a chart recorder (Kipp & Zonen® BD50, The Netherlands).

III. *Recommended Procedure*

Using the FI manifold shown in Figure 1, a 150 µL sample or standard solution containing tetracycline, chlortetracycline or oxytetracycline was injected into ammonium iron (III) sulfate (500 µg/mL) in 5.0×10^{-3} mol/L nitric acid (HNO_3) with an optimum flow rate of 4.0 mL/min. Subsequently, the sample zone flowed through the 75 cm in length of reaction coil, where the complexation occurred. The absorption was monitored by the detector at 423 nm and the FI signal was recorded on a chart recorder.

IV. *Sample Preparation*

The contents within the pharmaceutical capsule were 250 mg tetracycline hydrochloride™ (tetracycline hydrochloride) and 250 mg aureomycin™ (chlortetracycline hydrochloride). The powder of twenty capsules of each drug was weighed individually, then ground and mixed well. An amount of each drug equivalent to one capsule was accurately weighed and dissolved in water by sonication in a 250 mL volumetric flask and diluting with water to 250 mL. For 50 mg/mL oxytetracycline hydrochloride for injection™ (oxytetracycline hydrochloride for injection), transfer an accurately measured volume of injection, equivalent to 250 mg of oxytetracycline hydrochloride, to a 250 mL volumetric flask and diluting with water to 250 mL. The dissolved drug was filtered through Whatman No1 filter paper and diluted with water to volume to obtain the appropriate concentration for analysis.

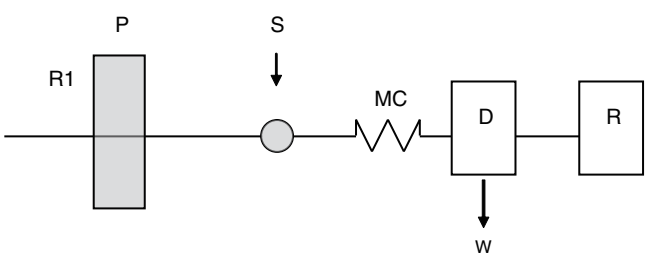

Figure 1. Proposed FI manifolds, R1, acidic iron (III) reagent; P, pump; S, tetracycline, chlortetracycline, oxytetracycline or sample solution; MC, mixing coils; D, detector; R, recorder; W, waste.

RESULTS AND DISCUSSION

Tetracycline, chlortetracycline or oxytetracycline reacted with iron (III) forming a soluble brown complex. The metal ligand ratio was found to be 1:2 using mole ratio and continuous variation method. The intense soluble brown complex formed by complexation between iron (III) and tetracycline, chlortetracycline or oxytetracycline led to the basis for the spectrophotometric determination of these drugs under the maximum absorbance at 423 nm.

The proposed flow system was developed and optimized by a univariate method. The variable by variable method was applied to select the optimum conditions for the flow injection spectrophotometric determination of tetracycline, chlortetracycline or oxytetracycline. For this method, the values of variable was changed while maintaining the other variables at their constant values. Afterwards, another variable was studied by maintaining that variable at its optimum value. The optimum values were selected from the appropriate absorption intensity.

I. *Effect of Iron (III) Concentration*

The effect of varying concentration of iron (III) solution between 100 - 900 µg/mL was examined. The appropriate peak height was obtained when the concentration of iron (III) in acidic solution was 500 µg/mL and was therefore chosen as the optimum concentration. However, further increase in iron (III) concentration caused the peak height to decrease gradually to 900 µg/mL (Figure 2).

II. *Effect of Mineral Acids*

The effect of 5.0×10^{-3} mol/L HNO_3, $HClO_4$, HCl, H_2SO_4 and H_3PO_4 in iron (III) solution was studied. The relative peak heights were 100.0, 61.5, 53.8, 46.2 and 23.1%, respectively. Yielding the peak height, HNO_3 was chosen for subsequent studies.

The concentration of HNO_3 in iron (III) solution was optimized. Various concentrations over the range 5.0×10^{-5} - 5.0×10^{-1} mol/L were investigated. It was found that the peak height increased with increasing HNO_3 concentration and reached a maximum peak height at

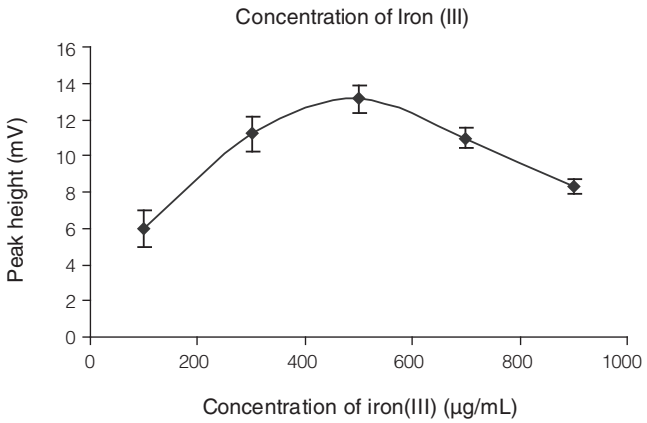

Figure 2. Effect of iron (III) concentration on the mean of peak height (n = 5) of 10 μg/mL.

5.0×10^{-3} mol/L, above which the peak height decreased. The 5.0×10^{-3} mol/L HNO_3 was used subsequently due to the stability of tetracycline under the acid media. The results received by the proposed method were in good agreement with those studied by Liawruangrath *et al*.[26] and Pena *et al*.[27].

III. *Effect of Mixing Coil Length and Injection Loop Volume*

This study was carried out at various mixing coil lengths between 25 and 125 cm and injection loop volumes between 50 and 250 μL on the complexation produced were investigated. It was found that the peak height increased with the mixing coil length up to 75 cm, and the mixing coil lengths of 25, 50, 75, 100 and 125 cm provided the peak height of 10.08, 11.10, 11.24, 7.32 and 6.72 mV, respectively.

With respect to the effect of the injection volume, it was seen that the peak height increased from 18.10 to 26.72 mV on increasing the injection volume from 50 to 250 μL. The maximum peak height was obtained at 150 μL. The most suitable mixing coil length and injection loop volume values for further use were 75 cm and 150 μL, respectively, at which the dispersion of FI system is very low[24].

IV. *Effect of Reagent Flow Rate*

The flow rate of iron (III) reagent was investigated based on the determination of tetracycline. The peak height increased from the flow rate of 2.0 to 4.0 mL/min and reached the maximum at 4.0 mL/min, while it decreased rapidly from the flow rate of 5.0 - 6.0 mL/min (Figure 3). As a compromise between sensitivity, sample throughput and reagent consumption which 4.0 mL/min iron (III) solution was regarded as the optimum flow rate, which disregarded the Schlieren effect of FI system[24].

V. *Analytical Characteristics*

Analytical characteristics for tetracycline, chlortetracycline and oxytetracycline were studied under the optimum conditions (Table 1).

VI. *Calibration Graph*

Under the optimum conditions, linear calibration graphs were obtained for 1.0 - 100.0 μg/mL of each tetracycline. Over the above concentration range, linear regression analysis of the peak heights in mV of each drug (y) versus each drug concentration (x) yield equations A, B and C for tetracycline, chlortetracycline and oxytetracycline, respectively. (A; $y = 1.29x - 0.46$, B; $y = 1.32x$

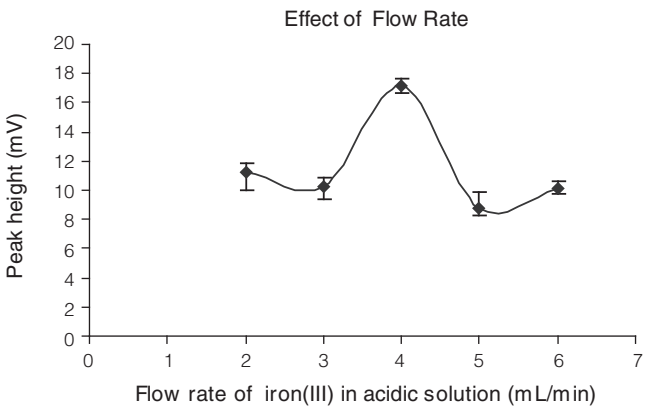

Figure 3. Effect of flow rate on the mean of peak height (n = 5) of 10 μg/mL.

Table 1. Variable ranges and optimum conditions for the determination of tetracycline, chlortetracycline and oxytetracycline

Parameter studied	Range studied	Optimum level
Wavelength (nm)	350 – 550	423
Iron (III) concentration (μg/mL)	100 – 900	500
Type of acid	HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$	HNO_3
HNO_3 concentration (mol/L)	$5.0 \times 10^{-5} - 5.0 \times 10^{-1}$	5.0×10^{-3}
Mixing coil length (cm)	25 – 125	75
Sample injection volume (μL)	50 – 250	150
Flow rate of iron (III) (mL/min)	2.0 – 6.0	4.0
Detector : UV-visible detection unit		
: Rise time (sec)	–	1.0
: Display	–	AU
: Range (AUFS)	–	0.20
: Temperature	–	Room temperature

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– 0.17, C; $y = 0.63x - 0.15$) The correlation coefficients were 0.995, 0.994 and 0.990 for tetracycline, chlortetracycline and oxytetracycline, respectively. It was found that chlortetracycline exhibited slightly higher sensitivity (defined as the slop of calibration graph) than tetracycline and oxytetracycline. The detection limit of each drug, the concentration of analyte that gives a signal different from the blank by an amount equal to three times the standard deviation of the blank signal (s/n = 3), was found to be 0.10 μg/mL. The quantitation limits (defined as ten times standard deviation) were 0.92 μg/mL.

VII. *Reproducibility and Accuracy*

The relative standard deviation of the proposed method (peak height in mV) calculated from 12 repeated injections of 10.0 and 25.0 μg/mL of tetracycline, chlortetracycline or oxytetracycline was 2.41%, 1.39%; 1.85%, 0.48% and 1.90%, 1.80%, respectively. Accuracy of the methods was determined by replicate injections (n = 12) of 150 μL aliquots of commercial drug sample solution spiked with the drug studied. After measurement of the peak height, the recovery of each spiked standard drugs were calculated. The percentage recoveries of 10.0 and 25.0 μg/mL (n = 12) of tetracycline, chlortetracycline or oxytetracycline were found to be 97.33%, 101.33%; 104.14%, 98.88% and 103.80%, 101.33%, respectively. It can be seen that the proposed method provided accurate results.

VIII. *Interferences*

The effect of various possible excipients (e.g. glucose, sucrose, lactose, maltose, cellulose, fructose and starch) were investigated with metal ions which are known to react with tetracycline[23]. Synthetic sample solutions containing 15 μg/mL of each drug and different concentration of the other compounds were tested, and the peak heights obtained were measured. It was found that glucose, sucrose, lactose, maltose, cellulose, fructose and starch had no effect on the determination of tetracyclines chlortetracycline or oxytetracycline even through they were present at a 10 or 20 times weight ratio to each drug. Most cations interfered and interestingly the most serious interference were from copper, zinc, lead, calcium, magnesium, manganese, iron (II), stannous and arsenic for chlortetracycline and oxytetracycline determination. However, only stannous and arsenic had serious effect on the determination of tetracycline (Table 2).

IX. *Application*

The proposed FI procedure was applied to the determination of tetracycline, chlortetracycline or oxytetracycline in commercial pharmaceutical formulations after appropriate sample pretreatment. The results were compared with those declared on the formulation labels and with those obtained by using the official method of pharmacopoeia[28]. The drug contents of tetracycline, chlortetracycline or oxytetracycline were found to be 276.0 ± 3.5, 250.1 ± 1.7 mg per capsule and 292.9 ± 2.7 mg per injection (n = 7) using this proposed method and 273.0 ± 2.0, 253.8 ± 2.3 mg per capsule and 301.9 ± 4.4 mg per injection (n = 7) using the official method. The results obtained by both methods were compared favorably by the student t-test at 95% confident level. Interestingly, this proposed method might be more appropriate for pharmaceutical capsule than pharmaceutical injection because of excipients which possibly present in formulations, including metal ions causing interference for oxytetracycline determination (Table 3).

Table 2. Effect of ions on the peak height of tetracycline, chlortetracycline and oxytetracycline, measured by comparing with 15 μg/mL of each drug

Interferences	Relative of peak height (%); *n* = 5		
(μg/mL)	Tetracycline	Chlortetracycline	Oxytetracycline
No Ions	100	100	100
Cu^{2+} (5)	-	8.28	11.31
Cu^{2+} (50)	-	6.02	9.61
Cu^{2+} (75)	62.96	-	-
Zn^{2+} (5)	-	9.25	13.76
Zn^{2+} (50)	-	6.02	8.67
Zn^{2+} (75)	56.52	-	-
Pb^{2+} (5)	-	8.06	13.67
Pb^{2+} (50)	-	6.67	6.97
Pb^{2+} (75)	57.69	-	-
Ca^{2+} (5)	-	8.17	15.46
Ca^{2+} (50)	-	6.67	8.67
Ca^{2+} (75)	57.32		
Mg^{2+} (5)	-	7.53	13.57
Mg^{2+} (50)	-	6.02	9.43
Mg^{2+} (75)	50.44	-	-
Mn^{2+} (5)	-	8.39	9.80
Mn^{2+} (50)	-	5.59	5.47
Mn^{2+} (75)	49.78	-	
Fe^{2+} (5)	84.70	8.17	14.51
Fe^{2+} (50)	55.78	5.38	5.28
Sn^{2+} (5)	7.32	7.53	15.08
Sn^{2+} (50)	5.56	6.24	5.66
As^{3+} (5)	6.00	8.82	16.78
As^{3+} (50)	5.86	6.02	8.48

Table 3. Accuracy of proposed FI method compared with standard determination method for tetracycline, chlortetracycline or oxytetracycline

No of Experiment	Tetracycline HCl 250 mg / Capsule		Chlortetracycline HCl 250 mg / Capsule		Oxytetracycline HCl 250 mg / 5 mL of Injection	
	FIA[1]	HPLC[2]	FIA[1]	HPLC[2]	FIA[1]	HPLC[2]
1	276.2	272.3	249.7	257.8	297.2	296.5
2	275.7	273.4	249.9	251.1	290.0	297.8
3	273.3	272.6	247.9	254.1	291.6	301.2
4	280.5	271.4	250.5	253.2	290.3	303.9
5	281.0	277.1	248.4	251.7	294.0	299.5
6	273.8	271.3	253.0	255.6	291.9	307.1
7	271.9	272.8	251.0	252.9	295.1	307.4
MEAN	276.0	273.0	250.1	253.8	292.9	301.9
S.D.	3.534	1.960	1.699	2.319	2.675	4.367
% R.S.D.	1.73	0.72	0.68	0.91	0.91	1.44

[1]FIA : Proposed Method

[2]USP : The United State Pharmacopeia, USP 26

CONCLUSIONS

The proposed FI spectrophotometric method has proven to be simple and sensitive for the determination of tetracycline, chlortetracycline and oxytetracycline. The calibration graph remains linear in the useful range for quantitation of each drug in pharmaceutical preparation. The detection limit of the method was more reasonable than those reported for spectrophotometry[3-5], fluorimetry[6,7], liquid chromatography[15], FI-electrochemical method[11-13], FI-capillary electrophoresis[19] and FI-chemiluminescence method[21,23]. This method is fast and economic, providing a good sample frequency of 70/h, and should be useful for routine analysis for tetracycline, chlortetracycline or oxytetracycline in pharmaceutical formulations. Thus, the determination of tetracycline, chlortetracycline or oxytetracycline by using this method might be applied for drug quality control and stability test.

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REFERENCES

1. Dax, S. L. 1997. Blackie Academic and Professional. In "Antibacterial Chemotherapeutic Agents". Chapter 4. London, UK.
2. Oka, H., Nakazawa, H., Harada, K.-I. and Macneil, J. D. 1995. AOAC International. In "Chemical Analysis for Antibiotics Used in Agriculture". Chapter 10. Arlington, U. S. A.
3. Salinas, F., Nevado, J. J. B. and Espinosa, A. 1989. Determination of oxytetracycline and doxycycline in pharmaceutical compounds, urine and honey by derivative spectrophotometry. Analyst 114: 1141-1145.
4. Alwarthan, A. A., Al-Tamrah, S. A. and Sultan, S. M. 1991. Spectrophotometric determination of oxytetracycline by flow injection. Analyst 116: 183-186.
5. Sultan, S. M., Suliman, F. O., Duffuaa, S. O. and Abu-Abdoun, I. I. 1992. Simplex-optimized and flow injection spectrophotometric assay of tetracycline antibiotics in drug formulations. Analyst 117: 1179-1183.
6. Poiger, H. and Schlatter, Ch. 1976. Fluorimetric determination of tetracyclines in biological materials. Analyst 101: 808-814.
7. Salinas, F., de la Peña, A. M. and Merás, I. D. 1990. Analysis of mixtures of doxycycline and oxytetracycline in pharmaceutical preparations by first derivative fluorimetry. Anal. Lett. 23: 863-876.
8. Gong, Z. and Zhang, Z. 1997. Determination of tetracyclines with a modified β-cyclodextrin based fluorosensor. Anal. Chim. Acta 351: 205-210.
9. Huang, C. Z., Liu, Y. and Li, Y. F. 2004. Microscopic determination of tetracycline based on aluminum-sensitized fluorescence of a self-orderd ring formed by a sessile droplet on glass slide support. J. Pharm. Biomed. Anal. 34: 103-114.
10. Ghandour, M. A. and Ali, A. M. M. 1991. Adsorptive

stripping voltammetric determination of tetracycline and oxytetracycline. Anal. Lett. 24: 2171-2186.
11. Couto, C. M. C. M., Lima, J. L. F. C., Conceição, M., Montenegro, B. S. M. and Reis, S. 1998. Tetracycline, oxytetracycline and chlortetracycline determination by flow injection potentiometry. J. Pharm. Biomed. Anal. 18: 527-533.
12. Gálvez, A. M., Mateo, J. V. G. and Calatayud, J. M. 1999. Study of various indicating redox systems on the indirect flow-injection biamperometric determination of pharmaceuticals. Anal. Chim. Acta 396: 161-170.
13 Palaharn, S., Charoenraks, T., Wangfuengkanagul, N., Grudpan, K. and Chilapakul, O. 2003. Flow injection analysis of tetracycline in pharmaceutical formulation with pulsed amperometric detection. Anal. Chim. Acta 499: 191-197.
14. Savage, A. L., Sarijo, S. H. and Baird, J. 1998. A novel screening method for tetracycline in milk combining sensitized-Eu (III) fluorescence and immunoaffinity techniques. Anal. Chim. Acta 375: 1-4.
15. Monser, L. and Darghouth, F. 2000. Rapid liquid chromatographic method for simultaneous determination of tetracyclines antibiotics and 6-epi-doxycycline in pharmaceutical products using porous graphitic carbon column. J. Pharm. Biomed. Anal. 23: 353-362.
16. Zhao, F., Zhang, X. and Gan, Y. 2004. Determination of tetracyclines in ovine milk by high performance liquid chromatography with a coulometric electric electrode array system. J. Chromatogr. A 1055: 109-114.
17. Charoenraks, T., Chuanuwatanakul, S., Honda, K., Yamaguchi, Y. and Chilapakul, O. 2005. Analysis of tetracycline antobiotics using HPLC with pulsed amperometric detection. Anal. Sci. 21: 241-245.
18. Anderson, W. C., Roybal, J. E., Gonzales, S. A., Turnipseed, S. B., Pfenning, A. P. and Kuck, L. R. 2005. Determination of tetracycline residues in shrimp and whole milk using liquid chromatography with ultraviolet detection and residue confirmation by mass spectrometry. Anal. Chim. Acta 529: 145-150.
19. Nozal, L., Arce, L., Simonet, B. M., Ríos, A. and Vacárcel, M. 2004. Rapid determination of trace levels of tetracyclines in surface water using a continuous flow manifold coupled to a capillary electrophoresis system. Anal. Chim. Acta 517: 89-94.
20. Han, H., He, Z. and Zeng, Y. 1999. Chemiluminescence determination of tetracyclines using a tris(2,2′-bipyridine)ruthenium(II) and potassium permanganate system. Anal. Sci. 15: 467-470.
21. Pena, A., Palilis, L. P., Lino, C. M., Silveira, M. I. and Calokerinos, A. C. 2000. Determination of tetracycline and its major degradation products by chemiluminescence. Anal. Chim. Acta 405: 51-56.
22. Lau, C., Lu, J. and Kai, M. 2004. Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile-hydrogen peroxide reaction. Anal. Chim. Acta 503: 235-239.
23. Townshend, A., Ruengsitagoon, W., Thongpoon, C. and Liawruangrath, S. 2005. Flow injection chemiluminescence determination of tetracycline. Anal. Chim. Acta 541: 105-111.
24. Calatayud, J. M. 1996. Taylor & Francis. In "Flow Injection Analysis of Pharmaceuticals." Chapter 1-2. London, UK.
25. Chang, W., Zhao, Y. and Ci, Y. 1992. Spectrofluorometric determination of tetracycline and anhydrotetracycline in serum and urine. Analyst 117: 1377-1378.
26. Liawruangrath, S., Liawruangrath, B., Watanesk, S. and Ruengsitagoon, W. 2006. Flow injection spectrophotometric determination of tetracycline in a pharmaceutical preparation by complexation with aluminium(III). Anal. Sci. 22: 15-19.
27. Pena, A., Carmona, A., Barbosa, A., Lino, C., Silveira, I. and Castillo, B. 1998. Determination of tetracycline and its major degradation products by liquid chromatography with fluorescence detection. J. Pharm. Biomed. Anal. 18: 839-845.
28. The United State Pharmacopeia, USP 26. 2003. pp. 438, 1378, 1792.