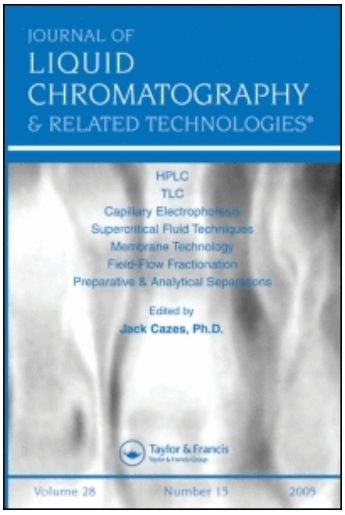

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Ticlopidine in Pharmaceutical Tablets by Flow Injection Analysis Using UV-Detection

Nafiz Ö. Can[a]; Göksel Altiokka[a]; Hassan Y. Aboul-Enein[b]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey [b] Department of Pharmaceutical and Medicinal Chemistry, National Research Centre, Dokki, Cairo, Egypt

To cite this Article Can, Nafiz Ö. , Altiokka, Göksel and Aboul-Enein, Hassan Y.(2008) 'Determination of Ticlopidine in Pharmaceutical Tablets by Flow Injection Analysis Using UV-Detection', Journal of Liquid Chromatography & Related Technologies, 31: 20, 3209 — 3218
To link to this Article: DOI: 10.1080/10826070802480230
URL: http://dx.doi.org/10.1080/10826070802480230

Journal of Liquid Chromatography & Related Technologies®, 31: 3209–3218, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802480230

Determination of Ticlopidine in Pharmaceutical Tablets by Flow Injection Analysis Using UV-Detection

Nafiz Ö. Can,[1] Göksel Altiokka,[1] and Hassan Y. Aboul-Enein[2]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey
[2]Department of Pharmaceutical and Medicinal Chemistry, National Research Centre, Dokki, Cairo, Egypt

Abstract: A precise and accurate flow injection analysis method for the quantification of ticlopidine in pharmaceuticals is described. An aqueous carrier stream, which was entirely prepared with water was chosen for the flow injection analysis. The method development was achieved by using a reference standard solution of ticlopidine at 3.19×10^{-6} M concentration, which was prepared in water. The solution was injected into the instrumental system at a flow rate of $1.0\,\text{mL} \cdot \text{min}^{-1}$ and signals were detected by a UV detector at 214.2 nm. The calibration curves of TP were linear in the concentration range of $1.59 \times 10^{-6} - 7.99 \times 10^{-5}$ M. The intra- and inter-assay precision was less than 1.3% relative standard deviation. The method exhibited good linearity with the correlation coefficients close to unity. The limit of detection and limit of quantization concentrations were found to be 8.91×10^{-8} and 2.70×10^{-7} M, respectively. The effects of the tablet excipients were insignificant at the 95% probability level. The calculated tablet contents were around 99%, which is in agreement with the ranges stated by pharmacopoeias.

Keywords: Flow injection analysis, Pharmaceutical analysis, Ticlopidine

Correspondence: Professor Hassan Y. Aboul-Enein, Department of Pharmaceutical and Medicinal Chemistry, National Research Centre, Dokki, Cairo 12311, Egypt. E-mail: enein@gawab.com

INTRODUCTION

Ticlopidine (TP), 4-[(2-Chlorophenyl)methyl]-4,5,6,7-tetrahydrothieno-[3,2-c]pyridine (Figure 1) is a potent and long acting thieno-[3,2-c]-pyridine derivative antithrombotic agent.[1] Pharmacological studies showed that TP is an effective inhibitor of adenosine diphosphate, collagen and adrenaline induced platelet aggregation.[2,3] TP is used therapeutically in the prevention of myocardial infarction and stroke, as well as in the treatment of transient ischemic attack, cerebral infarction and thromboembolism.[4–8] The superiority of TP to aspirin and dipyridamole as an antithrombotic agent was reported.[9,10]

A limited number of analytical techniques have been used for the determination of TP in drugs and biological samples. TP concentrations were determined by liquid chromatography[11–18] and gas chromatography[19–21] coupled with different types of detectors in these studies. The proposed method in the European Pharmacopoeia[22] is also a gas chromatographic method with flame-ionization detection. According to the best of our knowledge, no flow injection analysis (FIA) method has been reported so far for the determination of TP in pharmaceutical tablets. The methods reported in literature require solid phase extraction or relatively expensive equipments and reagents, which are not economically feasible for routine applications in pharmaceutical studies. The aim of this study is the development of a new analytical method for inexpensive, rapid and sensitive determination of TP in pharmaceutical tablets.

EXPERIMENTAL

Chemicals

HCl salt of TP (99%), which was used as a reference substance was purchased from Sigma-Aldrich Inc. (St. Louis, MO). Double distilled water used throughout the study was prepared, using all glass apparatus. Three

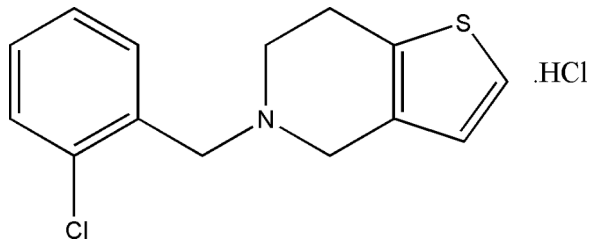

Figure 1. Chemical structure of TP.

different commercial formulations of TP present in the market, Agretik® 250 mg from Biokem İlaç Sanayi ve Ticaret A.Ş., Ticlid® 250 mg from Sanofi-Synthelabo İlaç A.Ş., and Ticlocard® 250 mg from Koçak Farma İlaç ve Kimya Sanayi A.Ş. were used as pharmaceutical forms of TP. All of the preparations were film coated tablets expressed as containing 250 mg TP each.

Apparatus

An HPLC system consisting a model LC-6A pump, a model SPD-10A UV-visible detector, and a model C-R7A Chromatopac integrator were used for FIA; reference solutions and samples were injected using a SIL-6B model auto injector (equipped with SCL-6B system controller) fitted with a 100 μL loop (all Shimadzu, Kyoto, Japan). The carrier solvent, 100% water was delivered at a flow rate of $1.0\,mL \cdot min^{-1}$ and the signal intensities were measured at 214.2 nm. A 1–6 model Sigma centrifuge and a Reax Top model Heidolph vortex were also used for the preparation of the samples.

Preparation of Solutions

The reference stock solution of TP (7.99×10^{-5} M) was prepared by dissolving an appropriate amount of TP in water at ambient temperature. Since TP is highly polar, dissolution and consequent dilutions were successfully made in water. Solutions were stored at 4°C and protected from light. Carrier solvent and reference solutions were filtered through 0.45 μm membrane filters before injection.

Analysis of Tablets

Twenty TP tablets were accurately weighed and the average weight of a tablet was calculated. The weighed tablets were finely powdered in a mortar. A sufficient amount of powder equivalent to the average weight of a tablet was accurately taken and transferred to a 100 mL volumetric flask. The volume was made up to 100 mL with water. The solution was magnetically stirred for 10 min. and then centrifuged at 3 g for 10 min. The supernatant of the solution was diluted with water and injected into the system. The procedure described here was employed for three commercial formulations of TP analyzed in the study.

RESULTS AND DISCUSSION

The appropriate carrier solvent, which is also suitable to dissolve TP was found to be 100% water and it was used throughout the study. This solvent did not cause any precipitation of TP. A solution of TP at the concentration of 3.19×10^{-6} M was used to determine the optimum conditions of FIA. Since the absorbance maximum of TP in water was observed at 214.2 nm, the detection was performed at this wavelength throughout the study. The instrumental parameters were investigated and set to provide the best peak symmetry and integration. Different flow rates were applied in the range of 0.1–3.0 $mL \cdot min^{-1}$ to determine the optimum flow rate. The relation of signals depending on the flow rate exhibited a parabolic curve. Linearity was observed only at the region of 1.0–1.6 $mL \cdot min^{-1}$ and the optimum flow rate was selected as 1.0 $mL \cdot min^{-1}$. Correlation between flow rate and TP signal fits to the equation [$S = 2.16 \times 10^{-5}\ F_R + 8.65 \times 10^{-7}$; $r = 0.994$] where S is the signal area and F_R is 1/[flow rate]. The injection volume as an important instrumental parameter was also studied. The injection volume varying from 5 to 50 μL was examined and regarding the peak morphology, 10 μL was employed as the injection volume. The equation expressing the flow rate – signal relation is [$S = 13189.1\ I_v - 57882.2$; $r = 0.996$] where S is the peak area and I_v is the injection volume.

Precision

The precision of the method was investigated by means of injection, intra-day (repeatability), and inter-day (reproducibility) precision. Injection performance of the auto injector was examined by employing repetitive injections of TP solution prepared at the concentration of 3.19×10^{-6} M to the system by 30 times. A relative standard deviation of 0.4% was calculated, exhibiting the sufficient injection precision for the assay. The intra-day precision was examined by injecting the TP solution (at 3.19×10^{-6} M) eight times in three consecutive days. The inter-day precision was calculated by pooling the intra-day results of the analysis. The results indicated that the method was quite precise, and the relative standard deviation was calculated to be 1.0 with a standard deviation of 453.9. Results are given in Table 1.

Linearity

Quantitative evaluation is based on the linear correlation between peak area and concentration. The linearity of the method was evaluated by

Table 1. Precision results of the assay

	Intra-day			Inter-day
Parameters	Day 1 (n = 8)	Day 2 (n = 8)	Day 3 (n = 8)	(k = 3; n = 24)
Mean	43435.3	43315.7	43147.9	43299.0
SD	268.5	503.6	550.8	453.9
RSD %	0.6	1.2	1.3	1.0
CL 0.05	186.0	348.9	381.6	181.6

SD: Standard deviation, RSD: Relative standard deviation, CL: Confidence limit (p = 0.05).

linear regression analysis using five different concentrations of TP. Signal area was chosen as the analytical response of the solute because high accuracy and linearity was gained with respect to peak height. The calibration range was between 1.59×10^{-6} M and 3.99×10^{-6} M presented with the equation of C (M) = $1.681 \times 10^{10} \times -8.681 \times 10^{3}$. The figure representing signal – concentration relationship is given in Figure 2.

The intercept is very low indicating the low noise of the real time analysis; additionally correlation coefficient close to unity (r = 0.9999). The values obtained showed good linearity and good agreement with Lambert-Beer's law. The intra-day and inter-day accuracy of the method was also examined. The characteristics of the calibration plots are summarized in Table 2.

The LOD and LOQ were determined by measuring the background response, and running ten blank solutions at maximum sensitivity. The LOD (S/N: 3.3) and LOQ (S/N: 10) of the LC method were calculated to be 8.91×10^{-8}M and 2.70×10^{-7}M, respectively.

Accuracy

A synthetic placebo tablet powder was prepared being composed of the ingredients of a commercial TP film coated tablet (citric acid, magnesium stearate, microcrystalline cellulose, povidone, starch, stearic acid, hydroxypropylmethyl cellulose, polyethylene glycol, and titanium dioxide)[23] to examine the effect of ingredients on the determination of TP. Approximately, 25 mg synthetic tablet powder weighed in 15 different tubes (1 = 3; n = 5); after addition of 1 mL of 7.99×10^{-5} TP solution, the mixture was vortexed for 5 min, left for incubation for 1 h., and then centrifuged at 3 g for 10 min. The solutions were made up to 10 mL with water, recentrifuged, and diluted 10 times to the concentration level of 10^{-6} M. Final TP concentrations were 1.59×10^{-6} for 50%, 3.19×10^{-6}

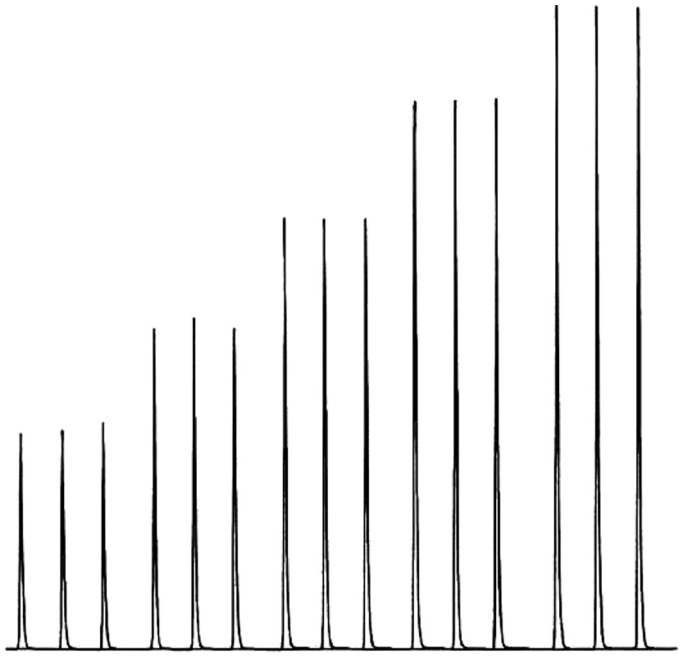

Figure 2. Concentration – signal relationship of TP between concentrations of 1.59×10^{-6} M and 3.99×10^{-6} M.

for 100% and 4.79×10^{-6} for 150% of tablet content. Furthermore, the same dilutions without placebo were prepared to find out their recovery. The accuracy of the method was evaluated as relative standard deviation (RSD %). The recovery results were given in Table 3.

Table 2. Main characteristics of the calibration plots

	Intra-day			Inter-day
Parameters	Day 1 (n = 5)	Day 2 (n = 5)	Day 3 (n = 5)	(k = 3; n = 15)
Slope, a	1.684×10^{10}	1.537×10^{10}	1.823×10^{10}	1.681×10^{10}
Intercept, b	-8.789×10^{3}	-3.923×10^{3}	-1.332×10^{3}	-8.681×10^{3}
Correlation coefficient, r	0.9997	0.9996	0.9996	0.9997
SD of regression equation, Sr	1.998×10^{3}	$3{,}2709 \times 10^{3}$	$4{,}1547 \times 10^{3}$	$8{,}1087 \times 10^{3}$
SD of slope, Se	5.329×10^{8}	$1{,}2945 \times 10^{9}$	$1{,}6443 \times 10^{9}$	$1{,}8529 \times 10^{9}$
CL (p = 0.05)	$\pm 1{,}28 \times 10^{9}$	$\pm 1{,}23 \times 10^{9}$	$\pm 1{,}56 \times 10^{9}$	$\pm 8{,}42 \times 10^{9}$

Table 3. Recovery results of the experiments

Added TP (M)	Found TP (M)	Recovery (%)	SD	RSD (%)
1.59×10^{-6}	1.58×10^{-6}	99.4	1.26	1.27
3.19×10^{-6}	3.17×10^{-6}	99.5	1.24	1.25
4.79×10^{-6}	4.75×10^{-6}	99.1	1.40	1.42

Table 4. Tablet assay of pharmaceutical tablets (n = 8 for each)

	Agretik® 250 mg	Ticlid® 250 mg	Ticlocard® 250 mg
Mean	248.2	249.6	248.8
Mean %	99.3	99.8	99.5
SD	1.81	1.69	1.54
RSD %	1.77	1.65	1.53
CL (p = 0.05)	1.59	1.33	1.24

SD: Standard deviation, RSD: Relative standard deviation, CL: Confidence limit (p = 0.05).

The adsorption or probable interaction that could affect the determination of TP was not observed for three groups. Recovery was higher than 99%, therefore it is concluded that the excipients do not interfere with the proposed method.

Application to Tablets

The tablets samples (Agretik® 250 mg, Ticlid® 250 mg, and Ticlocard® 250 mg), which was prepared as described before were analyzed at the specified conditions. Ten independent experiments were performed and the contents of the tablets were determined. The statistical evaluation of the assay is given in Table 4.

CONCLUSIONS

The main improvement of the present FIA method with respect to cited methods is the relatively low cost of the chemicals and apparatus used in the analysis procedure. The overall sensitivity is rather good, bearing in mind that the method uses no preconcentration or derivatization steps. FIA has certain advantages regarding simplicity, high sampling frequency, and low expense of reagents and samples, providing the technique suitable for quality control and routine analysis in many fields

such as environmental, food analysis, and biosensor technology.[24–30] FIA methods were also successfully applied in pharmaceutical assays carried out in the past.[31–37] This method has considerable time saving and has the superiority of high injection frequency which is very preferable for routine analysis. It also offers simple instrumentation and lower running cost. The simplicity of the carrier solvent makes the method extremely cost effective and simple if compared to the conventional chromatographic methods. The main disadvantage of this method is the unsuitable selectivity in the complex biological matrices such as plasma or urine. According to the results, it is clear that the procedure used in this study is reliable with high accuracy and precision. It could be concluded that the proposed FIA method described above is suitable for the routine analysis of TP in quality control laboratories.

REFERENCES

1. Thebault, J.J.; Blatrix, C.E.; Blanchard, J.F.; Panak, E.A. Effects of ticlopidine, a new platelet aggregation inhibitor in man. Clin. Pharmacol. Ther. **1975**, *18*, 485–490.
2. Nunn, B.; Lindsay, R. Effect of ticlopidine on human platelet responsiveness ex vivo: comparison with aspirin. Thromb. Res. **1980**, *18*, 807–813.
3. Saltiel, E.; Ward, A. Ticlopidine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in platelet-dependent disease states. Drugs **1987**, *34*, 222–262.
4. Hass, W.K.; Easton, J.D.; Adams, H.P.; Pryse-Phillips, W.; Molony, B.A.; Anderson, S.; Kamm, B. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine aspirin stroke study group. New Engl. J. Med. **1989**, *321*, 501–507.
5. Gent, M.; Easton, J.D.; Hachinski, V.C.; Panak, E.; Sicurella, J.; Blakey, J.A.; Ellis, D.J.; Harbison, J.W.; Roberts, R.S.; Turpie, A.G. The Canadian american ticlopidine study (CATS) in thromboembolic stroke. Lancet. **1989**, *8649*, 1215–1220.
6. Janzon, L.; Bergqvist, D.; Boberg, H.; Eriksson, I.; Lindgarde, F.; Persson, G. Prevention of myocardial infarction and stroke in patients with intermittent claudication; effects of ticlopidine. Results from STIMS, the Swedish ticlopidine multicentre study. J. Int. Med. **1990**, *227*, 301–308.
7. Ashida, S.I.; Abiko, Y. Mode of action of ticlopidine in inhibition of platelet aggregation in the rat. Thromb. Haemost. **1979**, *41*, 436–449.
8. Desager, J.P. Clinical pharmacokinetics of ticlopidine. Clin. Pharmacokin. **1994**, *26*, 347–355.
9. Tuong, A.; Bouyssou, A.; Paret, J.; Cuong, T.G. Metabolism of ticlopidine in rats: identification and quantitative determination of some its metabolites in plasma, urine and bile. Eur. J. Drug Metab. Pharm. **1981**, *6*, 91–98.

10. O'Brien, J.R.; Etherington, M.D.; Shuttleworth, R.D. Ticlopidine – an anti-platelet drug: effects in human volunteers. Thromb. Res. **1978**, *13*, 245–254.
11. Arnoux, P.; Sales, Y.; Mandray, M.; Lechat, P.; Berger, Y.; Cano, J.P. Quantitative high-performance liquid chromatographic, gas chromatographic, and gas chromatographic-mass spectrometric analysis of ticlopidine in baboon plasma after solid-phase extraction. J. Pharm. Sci. **1991**, *80*, 1092–1095.
12. Knudsen, J.B.; Bastain, W.; Sefton, C.M.; Allen, J.G.; Dickison, J.P. Pharmacokinetics of ticlopidine during chronic oral administration to healthy volunteers and its effects on antipyrine pharmacokinetics. Xenobiotica **1992**, *22*, 579–589.
13. Fujimaki, Y.; Tsumora, M.; Tachizawa, H. High performance liquid chromatographic method for the determination of ticlopidine in human serum. Yakugaku Zasshi. **1986**, *106*, 1143–1145.
14. Itoh, S.; Suzuki, S.; Ando, S.; Funatsu, Y.; Yamazaki, M.; Tanabe, K.; Sawanoi, M. Determination of ticlopidine in rabbit plasma by high-performance liquid chromatography. Chem. Pharm. Bull. **1987**, *35*, 1304–1307.
15. Dal Bo, L.; Verga, F.; Marzo, A.; Ambrosoli, L.; Poli, A. J. Chromatogr. B **1995**, *665*, 404–409.
16. Rona, K.; Ary, K.; Gachalyi, B.; Klebovich, I. Liquid chromatographic method for the determination of ticlopidine in human plasma. J. Chromogrt. B **1997**, *693*, 393–398.
17. Rona, K.; Ary, K.; Gachalyi, B. Validity of a liquid chromatographic (HPLC) method for the determination of ticlopidine in human plasma. Acta Pharm. Hung. **1997**, *67*, 25–29.
18. Furlan, G.; Malusa, N.; Strohmayer, A.; Klugmann, F.B.; Decorti, G.; Candussio, L.; Klugmann, S. Optimised method for determination of ticlopidine in serum by high performance liquid chromatography. Farmaco **1996**, *51*, 747–751.
19. Shah, J.; Fratis, A.; Ellis, D.; Murakami, S.; Teitelbaum, P. Effect of food and antacid on absorption of orally administered ticlopidine hydrochloride. J. Clin. Pharmacol. **1990**, *30*, 733–736.
20. Pena, A.; Lino, C.; Silveira, M.I. Determination of ticlopidine in human plasma by capillary gas chromatography with ion trap detection. J. Pharm. Biomed. Anal. **2003**, *8*, 879–885.
21. Shah, J.; Teitelbaum, P.; Molony, B.; Gabuzda, T.; Massey, I. Single and multiple dose pharmacokinetics of ticlopidine in young and elderly subjects. Brit. J. Clin. Pharmacol. **1991**, *32*, 761–764.
22. European Department for the Quality of Medicines within the Council of Europe, Strasbourg, European Pharmacopeia, B.P. 907 F 67029 Strasbourg CEDEX 1, **1997**, 1645–1646.
23. TICLID® (ticlopidine hydrochloride) TABLETS Complete Product Information. http://www.rocheusa.com/products/ticlid/pi.html.
24. Saurina, J.; Hernández-Cassou, S. Quantitative determinations in conventional flow injection analysis based on different chemometric calibration strategies: a review. Anal. Chim. Acta. **2001**, *438*, 335–352.

25. Duneca, A.F.; Cheregia, M.; Calatayudb, J.M.; Mateob, J.V.G.; Aboul-Enein, H.Y. Flow injection methods of analysis for waters. II. Organic Pollutants. Crit. Rev. Anal. Chem. **2003**, *33*, 57–68.
26. Daneta, A.F.; Cheregia, M.; Calatayudb, J.M.; Mateob, J.V.G.; Aboul-Enein, H.Y. Flow injection methods of analysis for waters. I. Inorganic Species. Crit. Rev. Anal. Chem. **2001**, *31*, 191–222.
27. Aboul-Enein, H.Y.; Stefanb, R.I.; van Stadenb, J.F.; Zhangc, X.R.; Garcia-Campanad, A.M.; Baeyens, W.R.G. Recent developments and applications of chemiluminescence sensors. Crit. Rev. Anal. Chem. **2000**, *30*, 271–289.
28. Yebra-Biurrun, M.C. Flow injection determinations of artificial sweeteners: a review. Food Addit. Contam. **2000**, *17*, 733–738.
29. Hansen, E.H. Principles and applications of flow injection analysis in biosensors. J. Mol. Recognit. **1996**, *9*, 316–325.
30. Stefana, R.I.; van Staden, J.L.; Aboul-Enein, H.Y. Design and use of electrochemical sensors in enantioselective high throughput screening of drugs. A mini review. Comb. Chem. High Throughput Screen. **2000**, *3*, 445–454.
31. Altiokka, G.; Atkosar, Z.; Can, N.O. The determination of levofloxacin by flow injection analysis using UV detection, potentiometry, and conductometry in pharmaceutical preparations. J. Pharmaceut. Biomed. **2002**, *30*, 881–885.
32. Can, N.O.; Tuncel, M.; Aboul-Enein, H.Y. Determination of nabumetone in pharmaceutical formulation by flow injection analysis (FIA) with UV-detection. Pharmazie. **2003**, *58*, 22–24.
33. Can, N.O.; Altiokka, G. Determination of Azapropazone in its Pharmaceutical Form by HPLC and Flow Injection Analysis. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 857–869.
34. Altiokka, G.; Altiokka, M. Flow injection analysis of amlodipine using UV-detection. Pharmazie. **2002**, *57*, 500–500.
35. Aboul-Enein, H.Y.; Dal, A.G.; Tuncel, M. A validated method development for ketoprofen by a flow-injection analysis with UV-detection and its application to pharmaceutical formulations. Farmaco. **2003**, *58*, 419–422.
36. Al-Momani, I.F. Flow Injection Spectrophotometric Determination of the Antibacterial levofloxacin in tablets and human urine. Anal. Lett. **2006**, *39*, 741–750.
37. Zhang, X.H.; Jin, O.Y.; Zhai, S.; Huang, G.M.; Zha, H.C. Flow-injection with enhanced chemiluminescence detection of ofloxacin in human plasma. Luminescence. **2005**, *20*, 362–369.

Received June 24, 2008
Accepted July 31, 2008
Manuscript 6368