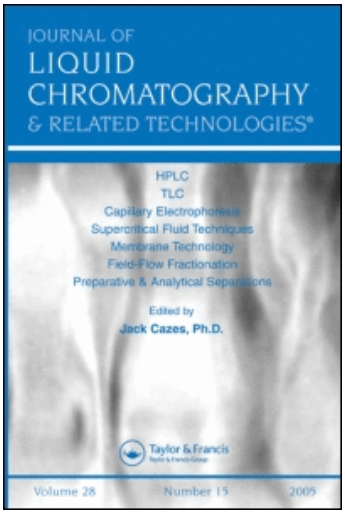

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Rapid HPLC and Direct Flow Injection Analysis Assay for the Determination of Trimetazidine HCl in Pharmaceutical Tablet Formulation

Göksel Altıokka[a]; Kevser Kırcalı[a]; Hassan Y. Aboul-Enein[b]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey [b] Pharmaceutical and Medicinal Chemistry Department, The Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Cairo, Egypt

To cite this Article Altıokka, Göksel , Kırcalı, Kevser and Aboul-Enein, Hassan Y.(2006) 'Rapid HPLC and Direct Flow Injection Analysis Assay for the Determination of Trimetazidine HCl in Pharmaceutical Tablet Formulation', Journal of Liquid Chromatography & Related Technologies, 29: 15, 2245 — 2255
To link to this Article: DOI: 10.1080/10826070600832921
URL: http://dx.doi.org/10.1080/10826070600832921

Journal of Liquid Chromatography & Related Technologies®, 29: 2245–2255, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600832921

Rapid HPLC and Direct Flow Injection Analysis Assay for the Determination of Trimetazidine HCl in Pharmaceutical Tablet Formulation

Göksel Altıokka and Kevser Kırcalı
Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

Hassan Y. Aboul-Enein
Pharmaceutical and Medicinal Chemistry Department,
The Pharmaceutical and Drug Industries Research Division,
National Research Center, Dokki, Cairo, Egypt

Abstract: A simple and sensitive high performance liquid chromatographic method (HPLC) and direct flow injection analysis method (FIA) are described for the determination of trimetazidine HCl (TMZ) in tablets. A mobile phase consisting of methanol:0.02 mol · L^{-1} potassium dihydrogen phosphate:0.005 mol · L^{-1} sodium dihydrogen phosphate (62:5:33 v/v/v) was used for the resolution of the compound on a reversed phase column for HPLC. For the FIA method; the best solvent system was found to be consisting of methanol:water (10:90 v/v). A flow rate of 1.2 mL · min^{-1} was pumped and active material was detected at 210 nm. Limit of detection (LOD) and limit of quantitation (LOQ) values were found to be 1.2×10^{-7} mol · L^{-1} and 3.5×10^{-7} mol · L^{-1}, respectively, for HPLC. LOD and LOQ values were found to be 2.35×10^{-7} mol · L^{-1} and 7.04×10^{-7} mol · L^{-1}, respectively for FIA. The results obtained from the analysis of TMZ tablet formulations were comparable using both methods.

Keywords: Trimetazidine HCl, High performance liquid chromatography, Flow injection analysis, Pharmaceutical analysis

Address correspondence to Hassan Y. Aboul-Enein, Pharmaceutical and Medicinal Chemistry Department, The Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Cairo 12311, Egypt. E-mail: hyaboulenein@yahoo.com

INTRODUCTION

Trimetazidine hydrochloride (TMZ), 1-(2,3,4-trimethoxypbenzyl)piperazine dihydrochloride regulates ionic and extracellular exchanges, correcting the abnormal flow of ion across the cell membrane caused by ischaemia, and preventing cellular oedema caused by anoxia.[1] The chemical structure of TMZ is shown in Figure 1. A number of methods have been reported for the determination of TMZ in biological fluids and pharmaceutical preparations. These include spectrophotometry,[2–5] high performance thin layer chromatography (HPTLC),[6] reversed phase liquid chromatography (RPLC),[7] gas chromatography-mass spectrometry (GC-MS),[8] chemiluminescence,[9] and voltammetry.[10]

Flow injection analysis is a new methodology characterized by its versatility, ease of automation, high sampling frequency, and minimum sample treatment prior to injection into the system. The FIA techniques have recently found wide applications, mainly due to a reduction of the analysis time and the consumption of reagents compared to conventional manual procedures.[11–13] They can also optimize the detection of analyte independently from the routine process occurring in the chromatographic column.[14]

The aim of this study is to develop a comparative analytical procedure for TMZ by using different analytical techniques, namely HPLC, FIA, and UV-spectrophotometry for the determination of TMZ in tablet formulations, and to compare the results of these validated methods.

EXPERIMENTAL

Apparatus and Chemicals

A Model LC 6A pump equipped with a 20 μL manual loop injector, a Model SPD-A10 UV variable wavelength detector, and a Model C-R7A integrator (all Shimadzu, Japan) were used for FIA measurements. A Shimadzu spectrophotometer (Model UV 2401 PC, Japan) was used to measure the absorbance in batch-wise operations. The analytical wavelength was 210 nm, and the area under the curve (AUC) was used for the calibration and measurement. A Model WTW Multiline P4 Universal pH-meter with a Sen-Tix 92T pH electrode was employed for pH measurements. For the chromatography, the HPLC system consisted of a Model Spectra System SCM 1000 degasser,

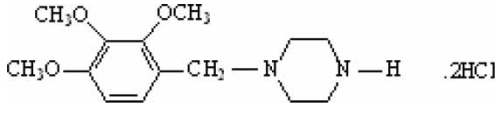

Figure 1. Chemical structure of TMZ.

Spectra System P1000 isocratic pump, Spectra System SN4000 connecter, Spectra System UV6000LP diode array detector (Thermo Finnigan, USA). The analyte peaks were resolved at the ambient temperature on a Phenomenex Nucleosil C18 (150 × 4.6 mm I.D.; particle size 5 μm) column. The volume injection loop was 20 μL. The data were collected and analyzed with Chrom Quest™ 4.0 HPLC database system on a IBM Pentium IV computer.

Standard TMZ (99.8%, purity) and tablets (Vastarel®) containing 20 mg of active material were kindly supplied by Servier Medicine and Investigation A.Ş. Istanbul, Turkey. Other chemicals were of analytical grade (E. Merck, Germany).

Preparation of Standard Solutions of TMZ for UV

A stock solution of TMZ (9.78×10^{-4} mol · L^{-1}) was prepared using bidistilled water. Standard solutions in the concentration range of 4.89×10^{-6}–2.45×10^{-5} mol · L^{-1} were made from the stock solution.

Preparation of Standard Solutions of TMZ for FIA

A stock solution of TMZ (1.0×10^{-3} mol · L^{-1}) was prepared using bidistilled water. Standard solutions in the concentration range of 1.0×10^{-6} – 5.0×10^{-6} mol · L^{-1} were made from the stock solution. As a mobile phase, an aqueous solution of methanol:water 10:90 (v/v) was used. The buffer solutions were prepared using 1 mol · L^{-1} CH_3COONa (pH 1–6) and 1 mol · L^{-1} K_2HPO_4 (pH 7–12), and their pH values were adjusted using 1 mol · L^{-1} HCl or 1 mol · L^{-1} KOH.

Preparation of Standard Solutions of TMZ for HPLC

A stock solution of TMZ (1×10^{-3} mol · L^{-1}) was prepared using bidistilled water. Standard solutions in the concentration range of 1.0×10^{-6} – 5.0×10^{-6} mol · L^{-1} were made from the stock solution. As a mobile phase, a mixture of methanol: 0.02 mol · L^{-1} potassium dihydrogen phosphate: 0.005 mol · L^{-1} sodium dihydrogen phosphate (pH adjusted to 4.0 with *o*-phosphoric acid) in the ratio of (62:5:33, v/v/v) was used.

Preparation of Tablet Samples

For the analysis of the pharmaceutical tablet formulation, 10 tablets of TMZ (Vastarel®) were accurately weighed, the average weight of a tablet was calculated, and finely pulverized with a pestle in a porcelain mortar.

For the UV, a portion of the powder equivalent to 20 mg TMZ was weighed accurately, and transferred to a 100 mL volumetric flask with 40 mL of bidistilled water. The flask was placed in ultrasonic water bath for 20 minutes before completion to volume with bidistilled water. A sufficient amount of the solution was centrifuged at 5600 rpm for 10 minutes. The supernatant was properly diluted with bidistilled water prior to the analysis.

For the FIA, the standard TMZ equivalent to one tablet and 1 mL acetate buffer (1 $mol \cdot L^{-1}$, pH 2.03) was transferred to a 100 mL calibrated flask, which was magnetically stirred for 20 min. and made up to volume with bidistilled water. A sufficient amount of the solution was transferred into a tube and centrifuged for 10 minutes at 5600 rpm. The supernatant was diluted with distilled water to predetermined values and injected into the sample loop by means of a syringe.

For the HPLC, a portion of the powder equivalent to 20 mg TMZ was weighed accurately, and transferred to a 100 mL volumetric flask with 40 mL of bidistilled water. The flask was placed in an ultrasonic water bath for 20 minutes before completion to volume with bidistilled water. A sufficient amount of the solution was centrifuged at 5600 rpm for 10 minutes. The supernatant was filtered through the 0.45 μm nylon syringe filter (sigma) and then properly diluted with bidistilled water prior to the analysis.

RESULTS AND DISCUSSION

UV Spectrophotometric Method

The UV-spectrophotometric technique is used as a comparative technique for the HPLC and FIA analyses. A series of TMZ solutions was prepared in the range of 4.89×10^{-6} $mol \cdot L^{-1}$ and 2.45×10^{-5} $mol \cdot L^{-1}$ from the stock solution of 9.78×10^{-4} $mol \cdot L^{-1}$. The UV-spectrophotometric behavior of TMZ was examined and regression analysis was performed. The absorbance maxima of TMZ was determined by scanning the absorbance values of 1.5×10^{-5} $mol \cdot L^{-1}$ TMZ solution in the range of 200–400 nm by 0.1 nm intervals. Therefore, 210.0 nm was found to be suitable and effective for the UV-spectrophotometric determination, and the rest of the study was carried out at this wavelength.

The linear relationship between the absorbance and the concentration of TMZ was established over the examined concentration range (4.9×10^{-6} – 2.5×10^{-5} $mol \cdot L^{-1}$). The average regression equation ($n = 5$) was calculated by the method of least squares and found to be $A = 34156.0\ C\ (mol \cdot L^{-1}) + 0.0031$. The average correlation coefficient was 0.9999.

The LOD (S/N:3.3) and LOQ (S/N:10) values are calculated to be 1.6×10^{-7} $mol \cdot L^{-1}$ and 4.9×10^{-7} $mol \cdot L^{-1}$, respectively.

FIA Method

A solution of TMZ at the concentration of 3.0×10^{-6} mol · L^{-1} was used to determine the optimum conditions of FIA. Methanol-water based systems at different percentages (10–50%, v/v) were investigated as carrier phase for the FIA procedure. It was found that the optimum concentration of methanol, in view of the peak morphology, was 10% (v/v). To determine the optimum flow rate, different flow rates were applied in the range of 0.2–3.0 mL · min^{-1} and signals were recorded. The optimum flow rate was found to be 1.2 mL · min^{-1}.

The effect of pH on the signals was determined via buffering of TMZ solutions in the pH range of 1.0 to 12.0 using acetate phosphate buffers. The pH was preferably adjusted by buffering, instead of using HCl or NaOH solutions, to prevent the pH change because of solvent addition. Morphologically good peak areas versus the pH were obtained at around pH 2. The differences in the peak area were minimum pH values ranging from 1 to 3, as shown in Figure 2. The AUC (area under curve) values for TMZ show great differences between pH 4 and 6. Moreover, the AUC values for TMZ decreased between pH 7 and 12. Therefore, a pH 2.03 buffer was used for the determination.

The final concentration of the buffer was adjusted to 0.1 mol · L^{-1} for all solutions and samples were carried to the detector set at 210 nm wavelength by methanol:water (10:90, v/v) at a flow rate of 1.2 mL · min^{-1}.

The effect of TMZ concentration on the peak signals was examined. A series of solutions were prepared in the range of $1.0 \times 10^{-6} - 5.0 \times 10^{-6}$ mol · L^{-1}. The correlation between concentration and signals are given in Figure 3.

The chemical stability of TMZ in the reference solution has been studied for a period of 7 days being stored at 2–8°C in a refrigerator. The prepared solutions gave the same signals during a week time.

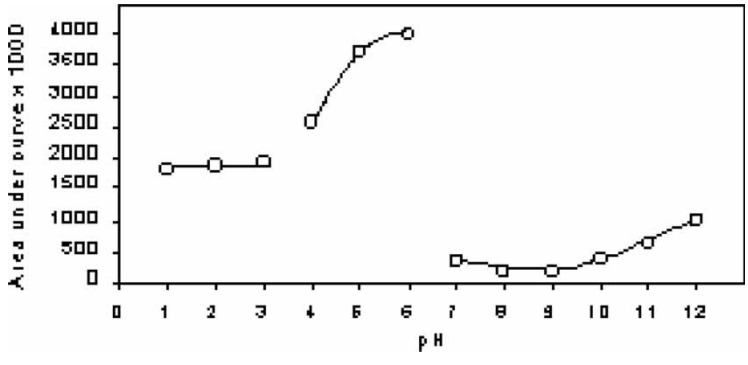

Figure 2. Variation in the AUC values of TMZ (9.8×10^{-7} mol · L^{-1}) in relation to the pH.

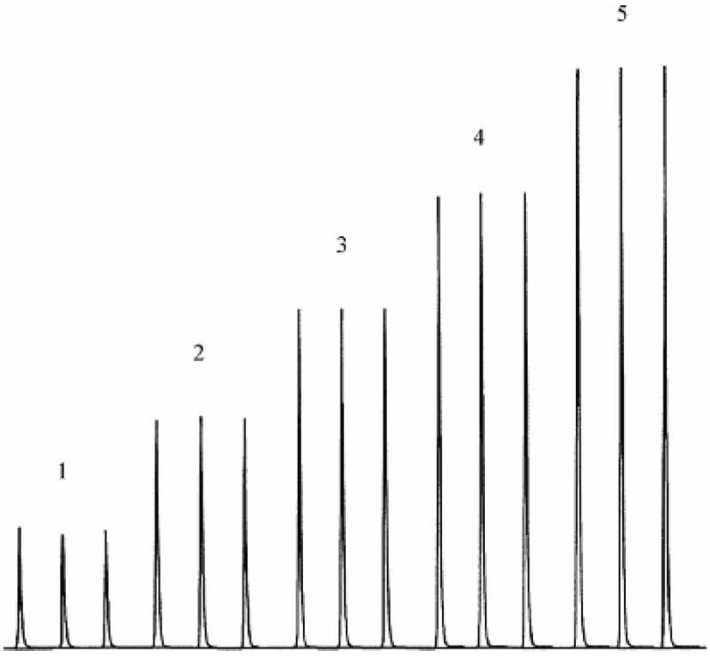

Figure 3. UV signal concentration of TMZ (1, 1.0×10^{-6}; 2, 2.0×10^{-6}; 3, 3.0×10^{-6}; 4, 4.0×10^{-6}; 5, 5.0×10^{-6} mol · L^{-1}).

Linearity and Accuracy

The signals area was chosen for the analytical response because high accuracy and linearity was gained with respect to peak area for FIA determination. The relationship between peak area and TMZ concentration was fitted to the equation, AUC $= 2.6 \times 10^{11}$ C (mol · L^{-1}) + 6229.3. with a high correlation coefficient, r = 0.9999.

The solutions were injected into the system for three subsequent days to validate the intra-day and inter-day linearity. The data obtained from the analysis are given in Table 1.

Table 1. Relationship between area under the curve (AUC) of FIA for TMZ

Parameter	Intra-day precision (k = 1, n = 8)	Inter-day precision (k = 3, n = 24)
x (mean)	1865816.6	1880664
SD	18488.6	32712.5
RSD%	0.99	1.74
CL($\alpha = 0.05$)	±15426.63	±13822.24

Precision

To examine the repeatability of the method, the TMZ solution at 3.0×10^{-6} mol · L^{-1} was injected to the FIA system for three days and the signals obtained were statistically evaluated. The data are given in Table 2.

The repeatability of the FIA method was highly precise as that of intra-day and inter-day precision, the relative standard deviation was 0.99% and 1.74%, respectively.

Sensitivity

The LOD (S/N = 3.3) and LOQ (S/N = 10) values of the method were calculated to be 2.35×10^{-7} mol · L^{-1} and 7.04×10^{-7} mol · L^{-1}, respectively.

HPLC Method

Various mobile phases had been tried during the method development of the HPLC analysis but the results were unsatisfactory. The mobile phase used consisted of a mixture of methanol:0.02 mol · L^{-1} potassium dihydrogen phosphate:0.005 mol · L^{-1} sodium dihydrogen phosphate (pH adjusted to 4.0 with *o*-phosphoric acid) in the ratio of (62:5:33, v/v/v). A Nucleosil C_{18} reversed-phase column (5 μm 150 × 4.6 mm) was used for the HPLC analysis, and the flow rate was kept constant at 0.9 mL · min^{-1}. The absorbances were recorded at 210 nm at ambient temperature. The signals were detected at 210 nm and the retention time of TMZ was found to be 4.85 min. The chromatogram obtained from the HPLC analysis of TMZ is shown in Figure 4. The responses of TMZ were evaluated by using a peak normalization procedure (peak area/retention time).

Table 2. Linearity and accuracy of the FIA method for TMZ

Parameter	Intra-day calibration (k = 1; n = 5)	Inter-day calibration (k = 3; n = 15)
Slope ± SD	$2.6 \times 10^{11} \pm 4.3 \times 10^{9}$	$2.7 \times 10^{11} \pm 3.1 \times 10^{9}$
Intercept	6229.4	10838.1
Correlation coefficient(r)	0.9999	0.9999
Slope ± CL($\alpha = 0.05$)	$2.6 \times 10^{11} \pm 4.1 \times 10^{9}$	$2.7 \times 10^{11} \pm 3.0 \times 10^{9}$

SD, Standard deviation.
CL, Confidence limit.
k, Number of the set.
n, Number of the sample.

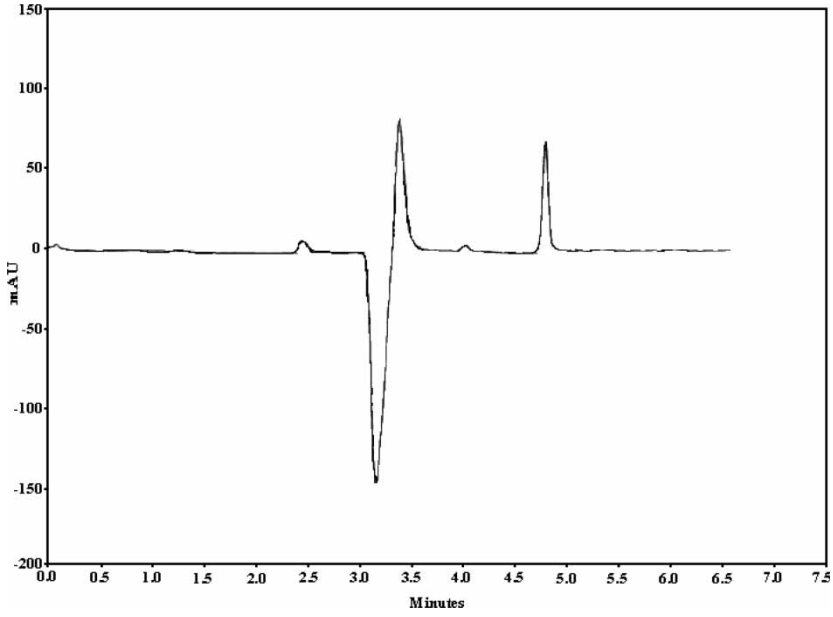

Figure 4. A typical chromatogram of TMZ (1.0×10^{-6} mol $\cdot$ L^{-1}).

Linearity and Accuracy

The linearity of the method was evaluated by linear regression analysis using five different concentrations of TMZ. The calibration range was between $1.0 \times 10^{-6} - 5.0 \times 10^{-6}$ mol $\cdot$ L^{-1}, presented by the equation of C (mol $\cdot$ L^{-1}) $= 1.7 \times 10^{11}$(PN) – 607.2. The correlation coefficient is close to unity (r = 0.9998). The values obtained showed good linearity and good agreement with Lambert-Beer's law. The intraday and inter-day accuracy of the method was also examined as shown in Table 3.

Table 3. Relationship between peak normalization values calculated from the data of HPLC analysis of TMZ

Parameter	Intra-day precision (k = 1, n = 8)	Inter-day precision (k = 3, n = 24)
X	1910332.1	1904472
SD	6698.6	12431.7
RSD%	0.35	0.65
CL($\alpha = 0.05$)	$\pm$5589.2	$\pm$5252.9

Precision

The repeatability of the method was examined by injecting TMZ solutions into the HPLC system for three consecutive days. The peak normalisation procedure was applied to minimize the external or internal factors that affect the analysis procedure. The results are given at Table 4.

As it is seen in Table 3, the repeatability of the HPLC method is precise where the relative standard deviation percentage of the repeatability is 0.65.

Sensitivity

The sensitivity of the method was presented by its LOD and LOQ. The LOD and LOQ were determined by measuring the background response, and running six blank solutions at maximum sensitivity. The LOD and LOQ of the method are calculated to be 1.2×10^{-7} mol $\cdot$ L^{-1} and 3.5×10^{-7} mol $\cdot$ L^{-1}, respectively.

Assay of Pharmaceutical Formulation

The proposed technique was applied to pharmaceutical dosage forms containing 20 mg TMZ. Batch-wise UV-spectrophotometry was chosen as a comparison for the determination of TMZ. The relationship between the absorbance (A) and the concentration (C) was found to be $A = 34156.0\ C\ (\text{mol} \cdot L^{-1}) + 0.0031$, $r = 0.9999$.

The results obtained from the HPLC and FIA methods were compared to that of the official UV method. Furthermore, the method was supported, except for the ruggedness and robustness parameters, by performing full validation parameters.

The validity of this method was examined by its application for the analysis of the active ingredient in the pharmaceutical tablets. The tablet excipients did not interfere with the analysis, since these inactive ingredients in the tablets showed no UV absorbance at a working wavelength of 210 nm. All results of the assay were evaluated statistically, as presented in Table 5. The results obtained from HPLC and FIA techniques were in agreement to that

Table 4. Linearity and accuracy of the HPLC method for TMZ in optimum conditions

Parameter	Intra-day calibration (k = 1; n = 5)	Inter-day calibration (k = 3; n = 15)
Slope $\pm$ SD	$1.7 \times 10^{11} \pm 3.4 \times 10^{9}$	$1.6 \times 10^{11} \pm 3.8 \times 10^{9}$
Intercept	-607.2	2292.8
Correlation coefficient(r)	0.9998	0.9997
Slope $\pm$ CL($\alpha = 0.05$)	$1.7 \times 10^{11} \pm 3.3 \times 10^{9}$	$1.6 \times 10^{11} \pm 3.6 \times 10^{9}$

Table 5. Assay results of TMZ in tablets[a]

Parameter	FIA	HPLC	Batch method (UV)
Mean/mg	20.17	20.08	20.34
n	8	8	8
RSD%	1.66	1.20	1.70
CL($\alpha = 0.05$)	± 0.28	± 0.20	± 0.29
F-Test of insignificant	2.815	1.447	$F_{0.05} = 3.79$ (table)
t-Test of insignificant	-1.393	-2.969	$T_{0.05} = 2.36$ (table)

[a]Each tablet contains 20 mg of TMZ.

obtained from UV-spectrophotometry. The results of the methods were compared to each other by common statistical tests at the probability level of 95%. The values of t- and F-tests of the experiments that were lower than the critical table values for t- and F-tests, state the insignificant difference between the methods.

CONCLUSION

To compensate for the missing of ruggedness and robustness, a UV-spectrophotometric method was used as a comparison method. The results obtained from the HPLC and FIA methods were compared to that of the official UV-spectrophotometric method. Furthermore, the method was supported, except for ruggedness and robustness parameters, by performing full validation parameters.

According to the results of the experiments performed under different analytical conditions, it was proven that all of the procedures used in this study were reliable with high accuracy and repeatability. It could be easily said that the HPLC, FIA, and UV-spectrophotometric methods mentioned in this study are suitable for the routine analysis of TMZ in quality control laboratories. The methods were applied to TMZ tablet formulations and the suitability of the methods to the pharmacopoeia was evaluated.

REFERENCES

1. Harpey, C.; Clauser, P.; Labrid, C.; Freyria, J.L.; Poirier, J.P. Cardiovasc. Drug Rev. **1989**, *6*, 292–312.
2. Issa, Y.M.; Abou-Attia, F.M.; Abdel-Gawad, F.M.; Abdel-Hamid, S.M. Anal. Lett. **2002**, *35* (3), 451–461.
3. Abou-Attia, F.M.; Issa, Y.M.; Abdel-Gawad, F.M.; Abdel-Hamid, S.M. Il Farmaco **2003**, *58*, 573–579.
4. Murthy, T.K.; Sankar, D.G.; Rao, R.Y. Asian J. Chem. **2002**, *14* (1), 169–172.
5. Darwish, I.A. Anal. Chim. Acta **2005**, *551* (1–2), 222–231.

6. Thoppil, S.O.; Cardoza, R.M.; Amin, P.D. J. Pharm. Biomed. Anal. **2001**, *25*, 15–20.
7. Thoppil, S.O.; Amin, P.D. J. Pharm. Biomed. Anal. **2001**, *25*, 191–195.
8. Fay, L.; Michel, G.; Goupit, P.; Harpey, C.; Prost, M.J. Chromatogr. Biomed. Appl. **1989**, *490*, 198–205.
9. Palilis, L.P.; Calokerinos, A.C. Anal. Chim. Acta **2000**, *413*, 175–186.
10. Ghonein, M.M.; Khashaba, P.Y.; Beltagi, A.M. J. Pharm. Biomed. Anal. **2002**, *27*, 235–241.
11. Al-Momani, I.F.; Awni, I.; Kilalil, H.S.; Esmadi, F. Anal. Lett. **1999**, *32* (15), 2977–2988.
12. Danet, A.F.; Badea, M.; Aboul-Enein, H.Y. Luminescence **2000**, *15* (5), 305–309.
13. Gonzales, F.; Tarin, P.; Maspoch, S.; Blanco, M. Arch. Pharm. **1988**, *321*, 725.
14. Altiokka, G.; Kircali, K. Anal. Sci. **2003**, *19* (4), 629–631.

Received March 20, 2006
Accepted April 18, 2006
Manuscript 6838