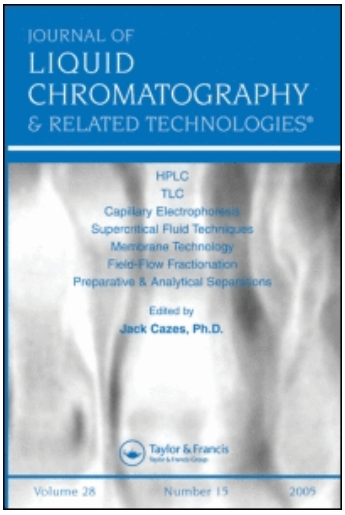

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Azapropazone in its Pharmaceutical Form by HPLC and Flow Injection Analysis

Nafiz Oncu Can[a]; Goksel Altiokka[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

To cite this Article Can, Nafiz Oncu and Altiokka, Goksel(2005) 'Determination of Azapropazone in its Pharmaceutical Form by HPLC and Flow Injection Analysis', Journal of Liquid Chromatography & Related Technologies, 28: 6, 857 — 869
To link to this Article: DOI: 10.1081/JLC-200051473
URL: http://dx.doi.org/10.1081/JLC-200051473

Journal of Liquid Chromatography & Related Technologies®, 28: 857–869, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200051473

Determination of Azapropazone in its Pharmaceutical Form by HPLC and Flow Injection Analysis

Nafiz Oncu Can and Goksel Altiokka
Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

Abstract: Azapropazone is an analgesic and anti-inflammatory agent that is used in the treatment of musculoskeletal and joint disorders, as well as in acute gout, because of its uricosuric properties. A simple and sensitive high performance liquid chromatographic method (HPLC), for the determination of azapropazone in its pharmaceutical form has been developed and validated. Azapropazone and indapamide (internal standard) were separated by a reversed phase column (Supelco Hypersil 5 μm, 150 × 4.6 mm ID, C_{18}) with a mobile phase consisting of K_2HPO_4 (0.1 M) and methanol (55 : 45, v/v) (at pH 7.0). The mobile phase was pumped at 1.2 mL min^{-1} flow rate then azapropazone was determined by ultraviolet detection at 251 nm. The method has an average analysis time of 5.81 min. for azapropazone. The flow injection analysis (FIA) was performed by using a carrier stream of ethanol : water (10 : 90, v/v) with a flow rate of 1.2 mL min^{-1}. The LOD and LOQ concentrations of azapropazone for the two methods were 2.77×10^{-8} M and 8.41×10^{-8} M for HPLC, 3.65×10^{-7} M and 1.10×10^{-6} M for FIA, respectively. The results obtained from the analysis of capsule samples using both methods were compared by common statistical tests. There was no significant difference observed between the methods.

Keywords: Azapropazone, Quantitative determination, Flow injection analysis, High performance liquid chromatography, Pharmaceutical application

Address correspondence to Goksel Altiokka, Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey. E-mail: galtiokk@anadolu.edu.tr

INTRODUCTION

Azapropazone (APZ), 5-dimethylamino-9-methyl-2-propylpyrazolo[1,2]$^{-}\alpha$][1,2,4] benzotriazine-1,3(2H)-dione dihydrate, is an analgesic and anti-inflammatory agent that is used in musculoskeletal and joint disorders. The chemical structure of APZ is shown in Figure 1.

Besides its analgesic and anti-inflammatory effects, APZ also has uricosuric properties and can be used in the treatment of gout.[1] APZ is well absorbed from the gastrointestinal tract.[2] It has been reported that it binds strongly to proteins and has a half-life ranging from 12 to 14 hours. APZ is excreted in the urine as an unchanged drug.[3] It was calculated that an average of 0.8 mg APZ was excreted in breast milk in a 12-hour period.[4] It is also reported that photosensitivity occurred during treatment with APZ.[5] The techniques used for the determination of APZ included HPLC,[6–10] GC,[11] TLC,[12,13] and NMR.[14]

The methods reported in literature require solid phase extraction or expensive reagents and equipments, which are not economically feasible for routine use in pharmacokinetic and pharmaceutical studies. The aim of this study was to develop inexpensive and simple procedures for determination of APZ in its capsule form by using HPLC, FIA, and UV-spectrophotometry. Investigations of the spectral behaviors of APZ with respect to UV-spectrophotometry, flow rate of mobile phase, pH, and peak area in relationship to concentration were examined. An internal standard (IS) was utilized to enhance the repeatability and sensitivity of the HPLC method. Indapamide was found to be a suitable IS for the method.

EXPERIMENTAL

Chemicals

Standard APZ and indapamide (IS) were obtained from Embil İlaç AŞ (Turkey) and Sanovel İlaç AŞ (Turkey), respectively. Prodisan® (300 mg) capsules (Embil İlaç AŞ) were used as the pharmaceutical form of APZ. All other chemicals were HPLC grade and were obtained from Merck KGaA, Darmstadt, Germany.

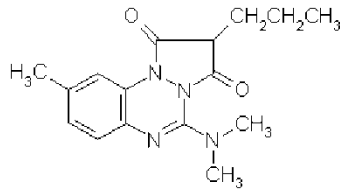

Figure 1. The chemical structure of APZ.

Apparatus

The spectrophotometric analysis of APZ was carried out by using a UV-2401PC model (Shimadzu) double beam UV-spectrophotometer and the results were processed by a UV/PC Personal Spectroscopy Software Version 3.7 program.

A WTW P4 Universal pH-meter cabled to a Sen-Tix 92T pH electrode (Germany) was employed for measuring and adjusting the pH of the solutions.

The centrifugation of the solutions to separate the insoluble particles was performed by a 1–6 model Sigma centrifuge.

The solutions were degassed using a B-220 model ultrasonic bath (Branson).

A liquid chromatograph pump (model LC-6A) with a 20 μL loop (Rheodyne-Cotati) was used for HPLC and FIA analysis. Signals were detected by a UV-VIS detector (model SPD-10A) and results were processed by a C-R7A Chromatopac integrator (all Shimadzu).

The separation of analytes for HPLC analysis was performed by using a 5 μm Hypersil C_{18} column, 150 × 4.6 mm ID (Supelco).

Standard Solutions

Standard stock solutions of APZ and Indapamide (IS) were prepared separately by dissolving 33.6 mg APZ and 36.6 mg IS, respectively, in ethanol to obtain 1.00×10^{-3} M concentrations. All of the subsequent dilutions for working standards were made with water. In order to minimize possible degradation of the analytes, they were protected from sunlight in opaque bottles and kept refrigerated.

Preparation of Capsule Samples

For the quantification of APZ in capsules, 10 APZ capsules were separately weighed, collected in a mortar, and the average weight of a capsule content was calculated. The amount of capsule powder corresponding to the average weight of a capsule content was accurately weighed and transferred to a 100 mL volumetric flask. After making up the volume with ethanol, the solution was magnetically stirred for 10 min. The solution was transferred to tubes and centrifuged for 10 minutes at 5600 rpm. For all of the determination techniques, the supernatant of the solution was collected, diluted with water, and the absorbance values for UV-spectrophotometry and the peak areas for the FIA and HPLC were recorded. The APZ amount of the capsule content was calculated by using the equation obtained from the analysis of a standard APZ reagent.

RESULTS AND DISCUSSION

HPLC Determination of APZ

The process for the development of the method for HPLC analysis included the testing of different types of mobile phases to obtain relatively short retention time for APZ. During this step, it was observed that methanol : water mobile phase containing less than 40% methanol did not give the effective mobility acceptable for analysis of APZ. Neither acetonitrile nor any other solvents were used, as the mobile phase proved adequate. Methanol : water (50 : 50, v/v) mobile phase was found to give the retention time of approximately 5 min. for APZ.

Several substances were tested to find a suitable IS for the analysis, and indapamide was found to be suitable, since it has similar solubility and has retention time closer to that of APZ. However, the addition of IS to the analysis process was found to be a problem because of signal overlapping of the two substances at the detection step. At this point, the necessity of a buffered mobile phase for the resolution of retention times of APZ and IS became clear. The phosphate and acetate buffered solutions in the form of methanol : buffer (v/v), at different pH values, were tested. The experiments showed that pH ranging between 4 to 7.5 gave the lowest retention of both APZ and IS. It was also observed that the retention time of APZ was not affected by pH, as much as that of IS, in the specified pH range, however, the main mobility factor for APZ was found to be methanol : buffer ratio in the mobile phase composition. Additionally, IS could not be detected at lower than pH 5, in spite of different ratios of methanol used in the mobile phase.

From these preliminary studies, the mobile phase containing K_2HPO_4 (0.1 M) : methanol (55 : 45, v/v) (at pH 7.0) was found to be the most suitable, and the flow rate was also determined to be 1.2 mL min^{-1} for the HPLC analysis. All mobile phases tested were degassed in an ultrasonic bath before analysis. IS concentration was kept constant at 2.00×10^{-6} M throughout the HPLC analysis. The signals were detected at 251 nm and the retention time of APZ and IS were found to be 5.81 and 6.76 min, respectively. The chromatogram obtained from HPLC analysis of APZ with IS is shown in Figure 2.

The chromatographic procedure was validated for linearity, precision, and sensitivity. The responses of APZ and IS were evaluated by using a peak normalization procedure (Peak normalization value (PNV) was calculated by dividing the area of the peak by its retention time). The validation of the method was achieved by using different concentrations of APZ versus a constant concentration of IS employing the ratio method, [analyte PNV]/[IS PNV] for every concentration as the response factor for the analyte. The signal normalization values were chosen instead of area response in order to

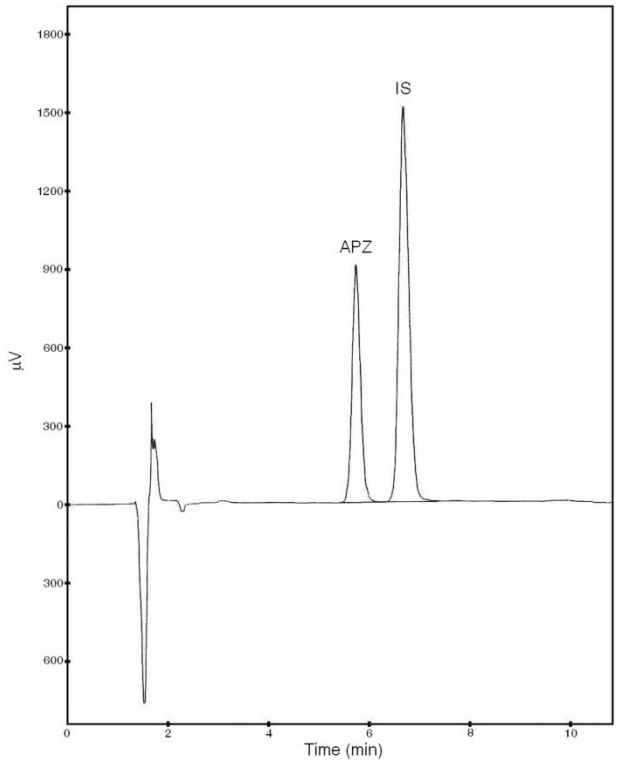

Figure 2. The chromatogram of APZ (5.65×10^{-7} M) and IS (2.00×10^{-6} M).

minimize the external or internal factors that affect the analysis, such as variation of ambient temperature, mobile phase composition, pH, etc.

Precision

The repeatability of the method was examined by injecting the solution consisting of APZ (5.65×10^{-7} M) and IS (2.00×10^{-6} M) into the HPLC system for three consecutive days. The results are given in Table 1.

The repeatability of the HPLC method was quite precise, and the relative standard deviation was calculated to be 1.504 with a standard deviation of 0.009.

Linearity and Accuracy

The linearity of the method was evaluated by linear regression analysis using six different concentration of APZ. The calibration range was between

Table 1. The statistical evaluation of HPLC analysis calculated from the data of APZ (5.65×10^{-7} M) and IS (2.00×10^{-6} M) using peak normalization procedure

	Intraday			
Parameter	Day 1 (n = 8)	Day 2 (n = 8)	Day 3 (n = 8)	Inter-day: all days (n = 24)
Mean	0.577	0.577	0.579	0.578
RSD %	1.558	1.684	1.441	1.504
$CL_{0.05}$	0.004	0.004	0.004	0.004

RSD: Relative standard deviation.
CL: Confidence interval ($\alpha = 0.05$).

1.96×10^{-7} M and 9.08×10^{-7} M presented with the equation of $C(M) = 1.03 \times 10^{6} \times (PNV) + 5.01 \times 10^{-3}$. The intercept is very small and the correlation coefficient 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. The evaluated data are given in Table 2.

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

Table 2. The intraday and inter-day accuracy and linearity of HPLC method was calculated using signal normalization values in the optimum conditions

	Intraday			
Parameter	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day: all days (n = 18)
a	986584.0	1034386.2	1082188.4	1034386.2
b	0.032	0.005	−0.022	0.005
r	0.9994	0.9997	0.9996	0.9998
Sr	0.02	0.02	0.02	0.05
Se	33286.86	31590.76	35529.70	51441.88
$CL_{0.05}$	31707.89	30922.25	33844.35	23376.74

a: slope.
b: intercept.
r: regression coefficient.
Sr: standard deviation of regression equation.
Se: standard deviation of the slope.
CL: confidence interval ($\alpha = 0.05$).

running six blank solutions at maximum sensitivity. The LOD (S/N = 3.3) and LOQ (S/N = 10) of the HPLC method were calculated to be 2.77×10^{-8} M and 8.41×10^{-8} M, respectively.

FIA Method of APZ Determination

FIA 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.[15–17] They can also optimize the detection of analytes independently from the routine process occurring in the chromatographic column.[18–20]

An HPLC apparatus has been used for the analysis in the flow injection method. The only difference is that the HPLC column has been removed from the HPLC apparatus. This has a great advantage in saving time and solvent for the analysis of a single substance because there is no need for the separation process.

A solution of APZ at the concentration of 9.99×10^{-6} M was used to determine the optimum conditions of FIA. Ethanol-water based systems at different percentages were tested as the carrier stream for the FIA procedure. However, there was no difference observed on peak area and height due to the variation of ethanol or water ratio. So, an ethanol : water (10 : 90, v/v) solution was chosen to be the carrier stream for the FIA. The presence of ethanol in the carrier composition was beneficial to prevent possible precipitations because of pH of the buffer or of any other solvents. To determine the optimum flow rate, different flow rates were applied in the range of 0.1–3.0 mL min^{-1} and signals were recorded. Linearity, depending on the flow rate, was observed at the region of 1.0–1.6 mL min^{-1} and 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 APZ solutions in the range of pH 1.0 to 11.0 by acetate or phosphate buffers. pH was preferably adjusted by buffering, instead of simply using HCl or NaOH solutions, to prevent the pH change because of solvent addition. The final concentration of the buffer was adjusted to 0.1 M for all solutions, and samples were carried to the detector set at 251 nm wavelength by EtOH : H_2O (10 : 90, v/v) at a flow rate of 1.2 mL min^{-1}. A significant difference was observed on the signal intensity due to pH variation of the solutions. The minimum change was observed between pH 2.0 and 6.0 (Figure 3); so, pH : 4.0 was employed as the working pH for the whole of the FIA study. It was clear that change in signal intensity should be as little as possible, in case of pH variation due to solvent addition or dilution.

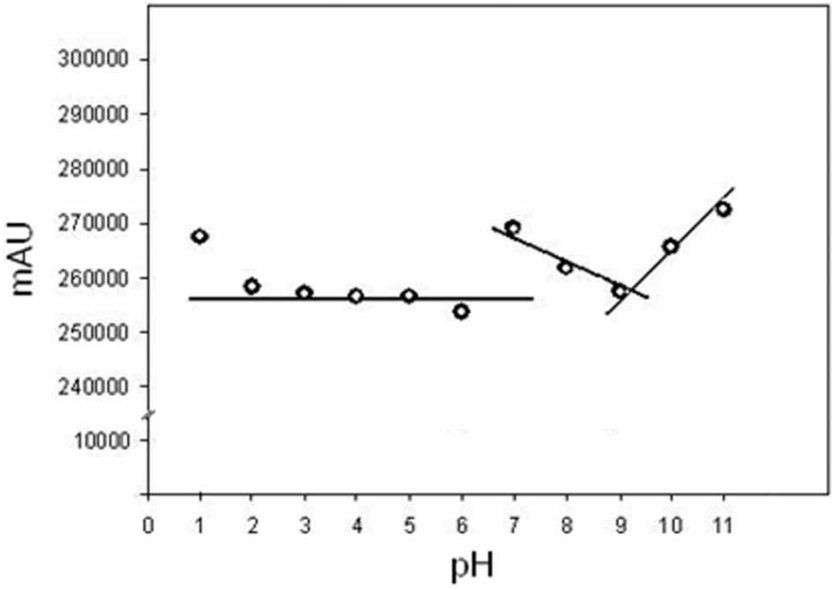

Figure 3. The pH-signal relationship for the FIA technique (n: 6).

To examine the effect of APZ concentration on the peak signals, a series of solutions were prepared in the range of $1.79 \times 10^{-5} - 6.99 \times 10^{-6}$ M. The correlation between concentration and signals are given in Figure 4.

Linearity and Accuracy

A signal area was chosen for analytical response because high accuracy and linearity was gained with respect to peak height for FIA determination. The relation between signal area and APZ concentration was fitted to the equation, $A = 2.42 \times 10^{10} \times C(M) + 1.60 \times 10^{4}$ with a high correlation coefficient, $r = 0.9998$. The solutions were injected into the system for three straight days to validate the intra-day and inter-day linearity. The data obtained from the analysis are given in Table 3.

Precision

To examine the repeatability of the method, APZ solution at 9.99×10^{-6} M was injected to the FIA system on three different days and the signals obtained were statistically evaluated. The data are given in Table 4.

The precision of the method has a RSD of 1.0%, which complies with the criteria proposed (RSD: $\leq$2.0%).

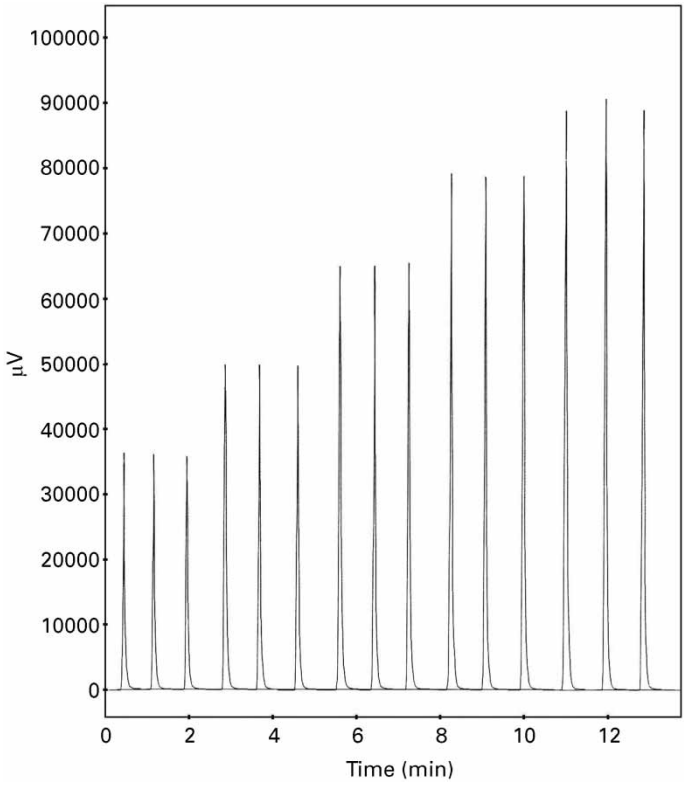

Figure 4. The FIA signals of APZ in the range of 1.79×10^{-5}–6.99×10^{-6} M.

Sensitivity

The LOD (S/N = 3.3) and LOQ (S/N = 10) values of the method were calculated to be 3.65×10^{-7} M and 1.10×10^{-7} M, respectively.

UV-Spectrophotometric Determination of APZ

The UV-spectrophotometric technique was used as a comparative technique for the whole analysis. A series of APZ solutions were prepared in the range of 6.99×10^{-6} M and 1.80×10^{-5} M by diluting the stock solution of 1.00×10^{-3} M with water. The UV-spectrophotometric behavior of APZ was examined and regression analysis was performed. The absorbance maxima of APZ was determined by scanning the absorbance values of 5.00×10^{-5} M APZ solution in the range of 200–400 nm by 0.1 nm intervals. Therefore, 251 nm was found to be suitable and effective for the

Table 3. The accuracy and linearity of the FIA method in the concentration range of 1.79×10^{-5} M–6.99×10^{-6} M obtained under optimum conditions

	Intra-day			
Parameter	Day 1 (n = 5)	Day 2 (n = 5)	Day 3 (n = 5)	Inter-day: all days (n = 15)
a	2.44×10^{10}	2.42×10^{10}	2.39×10^{10}	2.42×10^{10}
b	1.36×10^{4}	1.46×10^{4}	1.99×10^{4}	1.60×10^{4}
r	0.9997	0.9999	0.9999	0.9998
Sr	5.18×10^{3}	3.53×10^{3}	3.54×10^{3}	8.67×10^{3}
Se	5.84×10^{8}	3.99×10^{8}	4.00×10^{8}	5.64×10^{8}
$CL_{0.05}$	$\pm 5.56 \times 10^{8}$	$\pm 3.79 \times 10^{8}$	$\pm 3.80 \times 10^{8}$	$\pm 2.56 \times 10^{8}$

a: slope.
b: intercept.
r: regression coefficient.
Sr: standard deviation of regression equation.
Se: standard deviation of the slope.
CL: confidence interval ($\alpha = 0.05$).

UV-spectrophotometric determination and the rest of the study was carried out at this wavelength.

The relation between absorbance and concentration in the range of 6.99×10^{-6}–1.80×10^{-5} M was examined and the linearity stated in the equation $A = 35413.3 \times C(M) + 0.0001$ with a good correlation of $r = 0.9999$.

The LOD ($S/N = 3.3$) and LOQ ($S/N = 10$) values were calculated to be 3.07×10^{-6} M and 9.31×10^{-6} M, respectively.

Table 4. The intra- and inter-day repeatability of FIA determination of APZ

	Intra-day			
Parameter	Day 1 (n = 8)	Day 2 (n = 8)	Day 3 (n = 8)	Inter-day: all days (n = 24)
Mean	256415	255704	254713	255611
SD	1585.1	2973.0	3251.4	2679.6
RSD %	0.6	1.2	1.3	1.0
$CL_{0.05}$	1098.4	2060.1	2253.1	1072.5

SD: Standard deviation.
RSD: Relative standard deviation.
CL: Confidence limit ($\alpha = 0.05$).

Application to Capsules

The capsule samples (Prodisan® 300 mg capsules) prepared as described before, were analyzed using all of the techniques at the specified conditions. Eight independent experiments were performed and the content of the capsules was determined. No interference was observed originating from excipients or inactive ingredients. The assay of statistical evaluation is given in Table 5. The results obtained from HPLC and FIA techniques were fitted to that of UV-spectrophotometry; this was also proven by statistical evaluations. 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. Besides, the content of the capsules adhered to the official requirements stated in the pharmacopoeia.[21]

CONCLUSION

To compensate for the absence of ruggedness and robustness, a UV-spectrophotometric method was used as a comparison method. The results obtained from 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 a full validation process.

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, repeatability, and rapid and practical. It could be easily said that the HPLC, FIA, and UV-spectrophotometric

Table 5. The assay of APZ capsules (Prodisan® 300 mg capsules) (n = 8)

Parameter	HPLC	FIA	UV spectrophotometry
Mean%	101.53	102.11	100.60
SD	1.91	1.58	1.45
RSD%	1.87	1.54	1.44
$CL_{0.05}$	1.60	1.31	1.20
F test (p = 0.05)	1.73	1.19	3.79
T test (p = 0.05)	0.29	0.07	2.36

SD: Standard deviation.
RSD: Relative standard deviation.
CL: Confidence limit ($\alpha = 0.05$).

methods mentioned in this study are suitable for the routine analysis of APZ in quality control areas. The methods were applied to capsules that contain APZ and the suitability of the methods to the pharmacopoeia was evaluated.

ABBREVIATIONS

APZ	azapropazone
LOD	limit of detection
LOQ	limit of quantification
IS	internal standard
PNV	peak normalization value

REFERENCES

1. Fraser, R.C.; Davis, R.H.; Walker, F.S. Comparative trial of azapropazone and indomethacin plus allopurinol in acute gout and hyperuricaemia. J.R. Coll. Gen. Pract. **1987**, *37*, 409–411.
2. Jones, J.C. The pharmacology and pharmacokinetics of azapropazone—a review. Curr. Med. Res. Opin. **1976**, *4*, 3–16.
3. Parfitt, K. *Martindale's Extra Pharmacopoeia*; Pharmaceutical Press: London, 1999; 20.
4. Bald, R.; Bernbeck-Betthäuser, E.M.; Spahn, H.; Mutschler, E. Excretion of azapropazone in human breast milk. Eur. J. Clin. Pharmacol. **1990**, *39*, 271–273.
5. Olsson, S.; Biriell, C.; Boman, G. Photosensitivity during treatment with azapropazone. Brit. Med. J. (Clin. Res. Ed.). **1985**, *291*, 939.
6. Gikalov, I.; Kaufmann, R.; Schuster, O. Human pharmacokinetics of different i.v. doses of azapropazone (HPLC-determination) (author's transl). Arzneimittel-Forsch. **1982**, *32*, 423–426.
7. Kline, B.J.; Wood, J.H.; Beightol, L.A. The determination of azapropazone and its 6-hydroxy metabolite in plasma and urine by HPLC. Arzneimittel-Forsch. **1983**, *33*, 504–506.
8. Spahn, H.; Mutschler, E. HPLC determination of azapropazone in human plasma. Pharmazie **1983**, *316*, 42–47.
9. Rainsford, K.D. Distribution of azapropazone and its principal 8-hydroxy-metabolite in plasma, urine, and gastrointestinal mucosa determined by HPLC. J. Pharm. Pharmacol. **1985**, *37*, 341–345.
10. Ross, M.S.F. Chromatographic analysis of azapropazone and related benzotriazines. J. Chromatogr. A. **1977**, *131*, 448.
11. Leach, H. The determination of azapropazone in blood plasma. Curr. Med. Res. Opin. **1976**, *4*, 35–43.
12. Geissler, H.E.; Mutschler, E.; Faust-Tinnefeldt, G. On the determination of azapropazone from plasma by direct quantitative thin-layer chromatography (author's transl). Arzneimittel-Forsch. **1977**, *27*, 1713–1715.

13. Geissler, H.E.; Faust-Tinnefeldt, G.; Mutschler, E. Determination of azapropazone and 8-hydroxyazapropazone in urine by direct quantitative thin-layer chromatography (author's transl). Arzneimittel-Forsch. **1978**, *28*, 1430–1431.
14. Fenner, H.; Mixich, G. NMR studies on the molecular structure of azapropazone and interpretation of its pharmacokinetics and biotransformation. Arzneimittel-Forsch. **1973**, *23*, 667–669.
15. Al-Momani, I.F.; Awni, I.; Kilalil, H.S.; Esmadi, F. Spectrophotometric determination of cefadroxil in drug formulations using flow injection analysis. Anal. Lett. **1999**, *32*, 2977.
16. Danet, A.F.; Badea, M.; Aboul-Enein, H.Y. Flow injection system with chemiluminometric detection for enzymatic determination of ascorbic acid. Luminescence **2000**, *15*, 305.
17. Gonzales, F.; Tarin, P.; Maspoch, S.; Blanco, M. Flow injection amperometric determination of pharmaceuticals. Arch. Pharm. **1988**, *321*, 725.
18. 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. Pharm. Biomed. Anal. **2002**, *30*, 881.
19. 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.
20. Sener, E.; Tuncel, M.; Aboul-Enein, H.Y. Rapid determination of naproxen sodium in pharmaceutical formulations by flow injection analysis (FIA) using UV-detection. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26*, 401.
21. British Pharmacopoeia Commission. In *British Pharmacopoeia*; Her Majesty's Stationery Office: London, 1998; 1498.

Received July 12, 2004
Revised November 30, 2004
Manuscript 6463