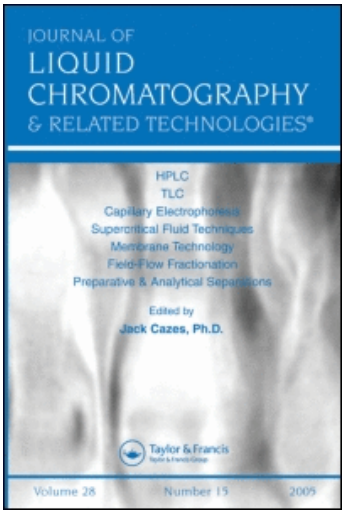

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A Simple and Rapid Reversed Phase High Performance Liquid Chromatographic Method for Quantification of Alprazolam and α-Hydroxyalprazolam in Plasma

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A SIMPLE AND RAPID REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR QUANTIFICATION OF ALPRAZOLAM AND α-HYDROXYALPRAZOLAM IN PLASMA

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ABSTRACT

A simple and rapid reversed-phase liquid chromatographic method for the determination of alprazolam and a-hydroxyalprazolam in plasma is described. Flunitrazepam was used as internal standard. Plasma samples were buffered with sodium borate and extracted with dichloromethane /n-pentane 4:6 v/v for 60 sec on a vortex apparatus. Extraction solvent was evaporated to dryness and extraction residues were reconstituted in the mobile phase. Samples were chromatographed on a 5μm Lichrospher RP-18 column (25cm x 4mm i.d) using acetonitrile/water 40:60 v/v as the mobile phase. The column effluent was monitored at 230nm. The lower limit of detection was 1ng/ml for alprazolam and a-hydroxyalprazolam while the lower limit of quantification was 2ng/ml for both compounds. Peak height and plasma

alprazolam or a-hydroxyalprazolam concentrations were linearly related from 2.5 to 40ng/ml. No potential sources of interference have been identified. The methodology was used to monitor plasma alprazolam concentrations in patients receiving two single doses of alprazolam ranging from 0.25 to 1.0mg each. Plasma alprazolam concentrations ranged from 4 to 27ng/ml at two hours post second dose.

INTRODUCTION

Alprazolam (8-chloro-1 methyl-6 phenyl-4H-s- triazolo [4,3-a] [1,4] benzodiazepine), is a relatively new member of a class of triazolobenzodiazepine compounds which is anxiolytic in humans (1,2) and may be effective in the treatment of depression (3-5) and panic disorders (6). The drug is rapidly absorbed and extensively metabolized and distributed (1). Hepatic biotransformation produces hydroxylated alprazolam derivatives and benzophenones, which are rapidly excreted in urine (1,7,8).

Alprazolam metabolites are not present in notable concentrations in human plasma (1,7,8). The major metabolite a-hydroxyalprazolam has significant pharmacological activity and is present at concentrations less than 5% of the corresponding alprazolam concentrations (8). Consequently, the measurement of the parent compound is most relevant to drug therapeutics being useful for clinical and toxicological studies (1,7,8).

Previously reported analytical methods for the quantification of alprazolam in plasma have been based on gas chromatography (9) and both normal-phase and reversed-phase high performance liquid chromatography (7,8,10-12). Benzodiazepine immunoassays for analysis of alprazolam in serum have also been reported (13,14).

This report describes a relatively simple, specific and accurate reversed-phase liquid chromatographic method, which allows quantification of alprazolam and a-hydroxyalprazolam in plasma. The method developed modifies previously described methods (7,8,10) in several aspects offering convenience and rapidity in analysis. Preliminary data suggests that the method is equally suitable for quantification of triazolam in plasma.

MATERIALS AND METHODS

Apparatus

Chromatography was performed with an HPLC system consisting of a JASCO (Japan Spectroscopic Co. LTD) Model 880 PU pump fitted with a model 880-02 Ternary Gradient Unit which was used under isocratic conditions on manual mode. The system fitted with a Model 7125 manual injector (Cotati, Rheodyne, California, U.S.A.) and a 50 µl sample loop. A Jasco Model 875 UV variable wavelength UV/VIS detector was operated at 230 nm. Samples were chromatographed on a 25 cm x 4mm (i.d.) Lichrospher RP-18 (MZ Analysentechnic D-6500 Mainz) reversed-phase column containing 5µm octadecyl (C_{18}) silane as the sorbent. A Hewlett Packard HP 3394A integrator was used to record chromatograms, at peak height mode (chart speed 0.5 cm/min).

A Millipore filtration system (Millipore, Bedford, M.A. USA) with type HV Millipore filters (pore size 0.45 µM) was used, for degassing mobile phase under vacuum.

A Fisher isotherm dry bath Model 145 was used for the evaporation of extraction solvents.

Reagents and chemicals

Analytical grade sodium borate was obtained from Serva. Acetonitril and water were HPLC grade (Lichrosolv^{R}) and were obtained from Merck. Dichloromethane and n-pentane were analytical grade and were obtained from Ferak Berlin. Alprazolam and a-hydroxyalprazolam were gifts from Upjohn (Kalamazoo MI, USA). Flunitrazepam was gift from Roche Pharmaceuticals (Nutley, NJ, USA).

Chromatographic Conditions

The mobile phase consisted of acetonitrile/water 40:60 v/v. A flow rate of 1.0 ml/min was used at ambient temperature, resulting in a pressure of about

127 kg/cm^2. Mobile phases were degassed by vacuum through filtration, after mixing. The column effluent was monitored at 230 nm with the detector set at 0.004 absorbance units full-scale.

Standards for calibration graphs

Stock standard solutions of alprazolam, a-hydroxy-alprazolam and flunitrazepam (internal standard) were prepared in methanol to give final concentrations of 1mg/ml. Standards stored at 4°C have been stable for 8 months.

Standard solutions of final concentration of 10µg/ml were prepared by diluting the stock standards 100-fold with distilled water.

An aqueous reference solution containing both alprazolam and a-hydroxyalprazolam to final concentration 1µg/ml was prepared from a 10µg/ml standard solution of each compound. Working solutions were prepared containing 25, 50, 100, 150, 200, 250, 300, 350, 400ng/ml of alprazolam and a-hydroxyalprazolam by appropriate dilutions of the reference solution with water. Plasma standards for calibration curves were prepared by spiking 1.0ml aliquots of pooled drug free plasma with 100µl of the above mentioned working solutions, to make alprazolam and a-hydroxyalprazolam plasma standards ranging from 2.5 to 40ng/ml.

A working solution of internal standard (200ng/ml) was prepared by dissolving 200µl of a 10µg/ml aqueous solution into 10ml of distilled water.

Calibration graphs of the recovered standards were prepared for each day of analysis to establish linearity and reproducibility of the HPLC system. Graphs were constructed of the peak-height ratio of each compound to internal standard against drug concentration.

Extraction procedure

In 10-ml glass conic tube with glass stopper, 1.0ml of plasma, 100µl of internal standard aqueous solution 200ng/ml (20ng),and 0.5 ml of solution of sodium borate

buffer (pH 9.3) were added and mixed briefly. Each sample was extracted with 5.0ml of dichloromethane/ n-pentane (4:6 v/v) on vortex for 60sec at speed 4 (Vortex - Genie, Model K-550 GE,Scientific Ind. Spingfield Mass 01103). The sample tube was centrifuged for 5min at 2000rpm. The upper (organic) layer was then transferred into a 10-ml conical glass tube and evaporated to dryness in a 50°C dry bath under a gentle stream of nitrogen. The residue was reconstituded in 100 µl of mobile phase, and an aliquot of about 70µl was injected onto the HPLC system.

Analytical variables

Recovery was calculated at 10,20,35 ng/ml spiked plasma samples by comparing the peak heights from extracted samples with those obtained from a direct injection of the corresponding unextracted standards dissolved in mobile phase.

Within run and between run precision was determined by extracting plasma supplemented with alprazolam and a-hydroxyalprazolam to 20ng/ml.

Assay versatility

The method was also evaluated for another triazolobenzodiazepine, triazolam. Quantification of triazolam in plasma, using flunitrazepam as internal standard, was also achieved.

In vivo study.

Eighteen female patients aged 35-50 years old were included in the study. All patients were programmed for surgical procedures due to benign gynecological diseases and were under good physical condition (scale ASA I,II). None of them appeared any history of psychiatric or other organic diseases and they were not receiving any other medication. Two single oral doses of Xanax^{R} brand of alprazolam (Upjohn) were administered to the patients late at night the day before operation and

early in the morning, before entering the surgery. From all patients blood samples were collected prior to first dose to serve as blank samples (at 11a.m.). The first dose of alprazolam was administered at 10p.m.; next morning at 6 a.m patients received the second dose of alprazolam. Blood samples were drawn two hours after the second dose (8a.m) by venipuncture of an antecubical vein, in a fasting state and were collected in unstoppered tubes containing sodium heparin as anticoagulant. Samples were centrifuged immediately after collection and plasma stored at -20°C until analysis. Patients included in the study were divided in 4 groups according to the dosage regimens administered:

1st Group (2 patients): Two single doses of 0.25mg alprazolam.

2nd Group (6 patients): Two single doses of 0.5mg alprazolam.

3rd Group (5 patients): Two single doses of 0.75mg alprazolam.

4th Group (5 patients): Two single doses of 0.5mg and 1.0mg alprazolam respectively .

RESULTS

Retention times for a-hydroxyalprazolam, alprazolam and flunitrazepam (internal standard) were 5.4, 7.8, and 10.1 minutes, respectively. Figure 1 shows a chromatogram obtained from a direct injection of an aqueous test solution containing 200ng/ml of each of the above mentioned compounds (injected volume 50µl corresponding to 10ng of each compound). Figure 2 shows chromatograms obtained from an extracted drug free plasma (a) and an extracted drug free plasma supplemented with a-hydroxyalprazolam, alprazolam and internal standard to 20ng/ml each (b). Figure 3 shows chromatograms obtained from extracted plasma of patients receiving two single doses of alprazolam.

No interfering peaks were observed in several samples of drug free plasma.

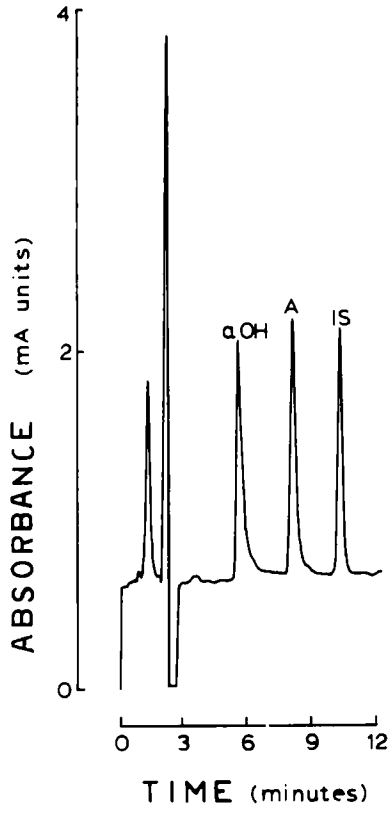

Figure 1:Chromatogram obtained from a direct injection of an aqueous test solution containing 200ng/ml of alprazolam(A), a-hydroxyalprazolam (aOH) and the internal standard- flunitrazepam (I.S).(Injected volume 50µl, corresponding to 10ng of each compound).

Recovery

Extraction recovery data from plasma samples supplemented with alprazolam and a-hydroxyalprazolam to 10,20,35 ng/ml are referred in Table 1 (means of five experiments). The metabolite a-hydroxyalprazolam was extracted in less extent (about 70%) in relation with alprazolam and the internal standard. Since alprazolam metabolites are not present in notable concentrations

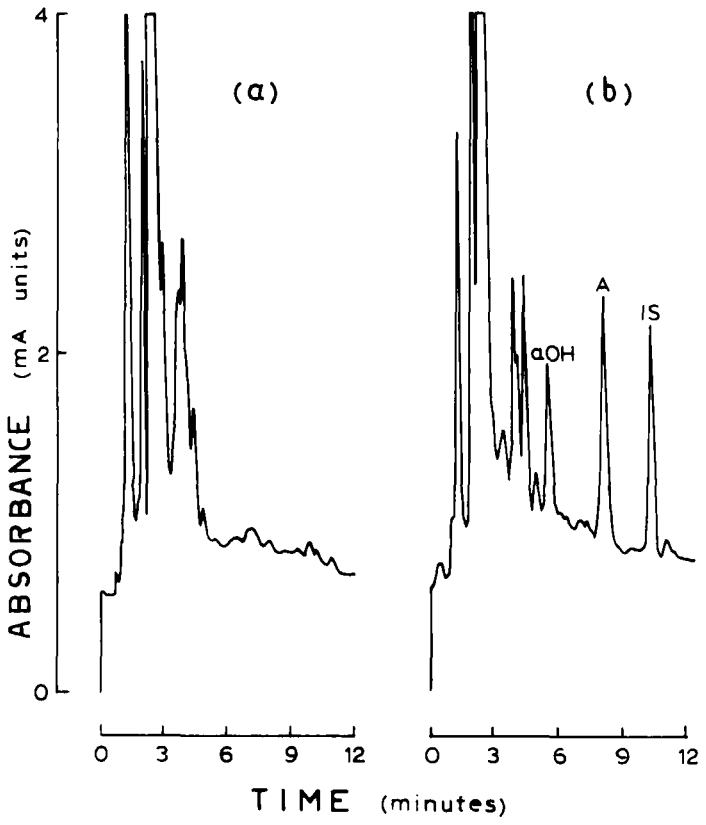

Figure 2:Chromatograms obtained from an extracted drug free plasma (a) and an extracted drug free plasma supplemented with alprazolam (A),a-hydroxyalprazolam(aOH) and internal standard-flunitrazepam(I.S) to 20ng/ml each (b).

in human plasma, the lower recovery of a-hydroxyalprazolam cannot be considered as a disadvantage of the method.

Linearity

The peak height ratios for alprazolam / internal standard and a-hydroxyalprazolam / internal standard were linearly related to plasma concentrations of

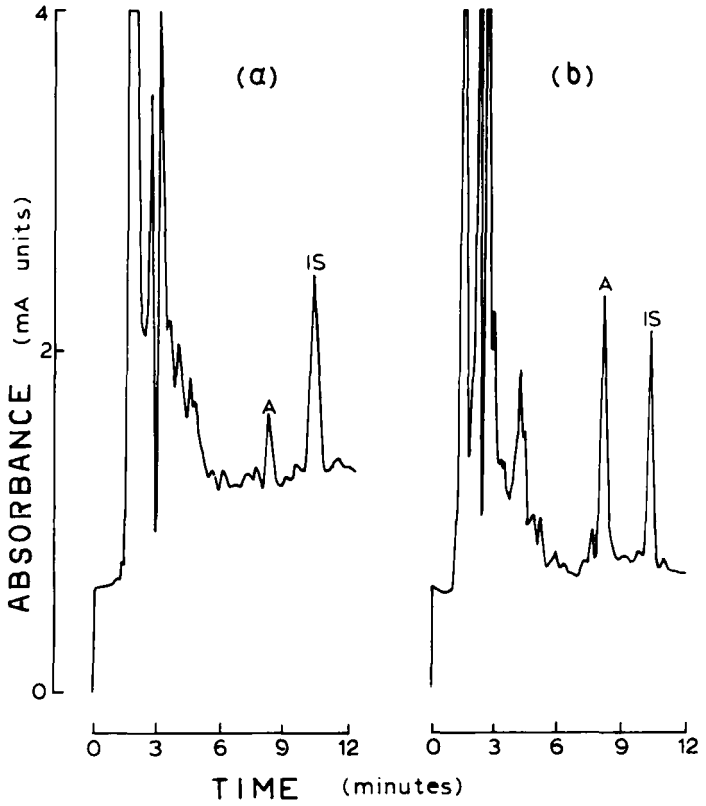

Figure 3:Chromatograms obtained from extracted plasma samples of patients receiving two single doses of alprazolam, collected 2 hrs post second dose.Alprazolam(A), internal standard- flunitrazepam (I.S).
(a) First dose: 0.5 mg alprazolam
Second dose: 0.5 mg alprazolam
Plasma alprazolam concentration: 7.0 ng/ml (Patient D,Table 3)
(b) First dose: 0.5 mg alprazolam
Second dose: 1.0 mg alprazolam
Plasma alprazolam concentration: 20.8 ng/ml(Patient P,Table 3)

alprazolam and a-hydroxyalprazolam, respectively, from 2.5 to at least 40ng/ml.

The slopes of 15 calibration curves of alprazolam in plasma, prepared over a period of two months, had a CV of 5.98%. The average regression equation was: $y = 0.053 x + 0.027$, where y = peak height ratio of

TABLE 1.

Extraction recoveries and Coefficient of variation(CV) of a-hydroxyalprazolam, alprazolam and flunitrazepam from spiked plasma samples.

	a-hydroxyalprazolam		Alprazolam		Flunitrazepam	
Spiked plasma standards(ng/ml)	Recovery%±SD	CV	Recovery%±SD	CV	Recovery%±SD	CV
10 (n = 5)	68.6±3.1	4.5	92.1±3.6	3.9	91.2±2.6	2.8
20 (n = 5)	71.0±2.5	3.5	90.5±3.6	4.0	92.1±2.8	3.0
35 (n = 5)	69.6±3.8	5.4	91.2±3.5	3.8	89.8±3.9	4.3

alprazolam / internal standard and x = plasma concentration of alprazolam (ng/ml). The correlation coefficients for each standard curve constructed invariably exceeded 0.998.

The slopes of 15 calibration curves for a-hydroxyalprazolam in plasma over a period of two months had a CV of 6.5%. The average regression equation was: y = 0.039 x + 0.04 where y = peak height ratio of a-hydroxyalprazolam / internal standard and x = plasma concentration of a-hydroxyalprazolam (ng/ml). Correlation coefficients for each standard curve exceeded 0.994.

The lower limit of detection was 1ng/ml for alprazolam and a-hydroxyalprazolam while the lower limit of quantification was 2ng/ml for both compounds.

Reproducibility and Accuracy

Within-run CV was 4.8% at 20ng/ml spiked plasma (n=10) with a mean concentration ± SD (ng/ml) of 18.84±0.90 (Relative Error, Er = - 0.058 and Relative Range Rr = 0.127).

Between-run CV was 5.2% for 20ng/ml spiked plasma (n=17) with a mean concentration ± SD (ng/ml) of 19.04 ± 1.0, over a period of 2 months.

TABLE 2.

Compounds studied for interference.

Lorazepam[a]	Diazepam[c]
Oxazepam[a]	Desmethyldiazepam[c]
Bromazepam[a]	Nitrazepam[c]
Chlorthiazide[a]	Imipramine[c]
Hydrochlorthiazide[a]	Haloperidol[c]
Perphenazine[a]	Chlorpheniramine[c]
Chlorpromazine[a]	Hexobarbital[c]
Thioridazine[a]	Amobarbital[c]
Triazolam[b]	Pentobarbital[c]
Chlordiazepoxide[b]	Diphenylhydantoin[c]
Carbamazepine[b]	Amitriptyline[c]
Caffeine[c]	Nortriptyline[c]

(a) = Not detected
(b) = Interfered, see text.
(c) = Detected but not interfered.

TABLE 3.

Plasma alprazolam concentrations of 18 patients included in the study.

Patient	1st oral dose (mg)	2nd oral dose (mg)	Plasma Alprazolam (ng/ml)
A	0.25	0.25	11.4
B	"	"	4.0
C	0.5	0.5	7.8
D	"	"	7.0
E	"	"	10.8
F	"	"	7.8
G	"	"	13.7
H	"	"	5.1
I	0.75	0.75	21.0
J	"	"	20.6
K	"	"	14.0
L	"	"	20.1
M	"	"	22.0
N	0.5	1.0	14.1
O	"	"	26.9
P	"	"	20.8
Q	"	"	9.1
R	"	"	20.4

Interferences

Twenty four compounds were studied for possible interference, including several drugs that might be administered to anxious or depressed patients (Table 2). Chlordiazepoxide produced peak between the peaks of alprazolam and internal standard; resolution was insufficient for quantification of alprazolam. Carbamazepine peak was overlapping with that of a-hydroxyalprazolam. Triazolam, another triazolobenzodiazepine, can not be separated from alprazolam by the described method but in clinical practice, it is not administered with alprazolam.

In vivo study

Table 3 shows alprazolam plasma concentrations in 18 patients taking two single doses of alprazolam ranging from 0.25 to 1.0mg each. The plasma concentrations of alprazolam were ranging from 4.0 to 26.9 ng/ml, at two hours post second dose.

DISCUSSION

A number of analytical methods, using high performance liquid chromatography, have been reported for quantification of alprazolam in plasma (7,8,10-12). Although these methods are sensitive and accurate, they are time consuming during the extraction procedure.

In this study several solvent mixtures were tested for the extraction of alprazolam, a-hydroxyalprazolam and flunitrazepam (internal standard) from plasma samples. Dichloromethane/n-pentane (4:6 v/v) was found suitable for the extraction; it appeared satisfactory extraction recovery (Table 1) and was rapidly evaporated at 50°C since both solvents of the mixture have very low boiling points. In previously reported methods (7,8,10) ethylacetate/heptane (10), toluene/isoamylalcohol (7) or toluene (8) have been used for the extraction, which require much longer time for the evaporation step. Furthermore, in the present study, plasma samples were extracted on a vortex apparatus for

only 60 sec, while previously reported methods (7,8,10) suggest extraction for 10-15 minutes on a rotator (7) or a reciptocating shaker (8,10). Therefore, it can be concluded that the method developed is more rapid and convenient in comparison with previously reported ones.

According to the published literature, alprazolam and a-hydroxyalprazolam can undergo ring opening under acidic conditions (10,15).Sodium borate buffer (pH 9.3) was used in the extraction procedure to adjust pH to maintain the closed ring structure (10). Extraction performed without pH adjustement, following a previously reported method (7), resulted in lower extraction recoveries.

In the method developed in this study late eluting peaks were not observed. Therefore a wash-out phase of the column between injections was not needed. In a previously described method (10), the column was washed between injections with 10ml of 70% acetonitrile in water to minimize the possibility of late eluting peaks and 10ml of mobile phase (acetonitrile/50mM potassium phosphate buffer pH 6, 30:70 v/v) were then pumped to re-equilibrate the column before next injection. This procedure prolongs time of analysis and it is not encountered in the present methodology.

Good chromatography (i.e peak shape, retention time) was achieved with acetonitrile/water 40:60 v/v mobile phase. The presence of phosphate buffer in the mobile phase, used in other methods (7,10) has been avoided. Thus,pH adjustement of the mobile phase as well as wash out of the column with water at the end of the day are not needed.Additionaly, analysis was performed at ambient temperature while other methods (7) include higher column temperature which is hazardous for the column.

Although the method has not been extensively used for the quantitation of triazolam, it appears to have sufficient specificity, sensitivity and precision for the quantitation of this triazolobenzodiazepine over a concentration range similar to that of alprazolam, using flunitrazepam as internal standard.

Alprazolam plasma concentrations in 18 patients included in this study, who received two single doses of alprazolam ranging from 0.25 to 1.0mg, were found to be between 4.0 to 26.9 ng/ml, at two hours post second

dose. These plasma levels are in accord with alprazolam plasma concentrations reported in relevant studies (7,9,10,16,17,18).

The metabolite a-hydroxyalprazolam was not detected in plasma samples of the patients included in the present study. This is consistent with previously published data (7), since monohydroxylated metabolites were not detected in a patient with plasma alprazolam concentration of 26.4ng/ml. Furthermore, a fatal poisoning from alprazolam overdose showed plasma alprazolam levels of 177ng/ml while the presence of alprazolam metabolites was not mentioned (19). Moreover, serum from a patient with alprazolam exceeding 300ng/ml (acute overdosage) demonstrated only trace amounts (less than 2.5ng/ml) of hydroxylated metabolites (7). Previous studies in healthy volunteers have not detected alprazolam metabolites in significant concentrations in serum after 1 or 2mg oral doses (9,17,18).

In conclusion, the analytical methodology developed in this report is simple, rapid, accurate, sensitive and specific. It can be used for monitoring plasma alprazolam concentrations in clinical studies. The present methodology provides also the possibility for quantification of a-hydroxyalprazolam in plasma; this is certainly of value for studies in other species.

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REFERENCES

1. Dawson, G.W., Jue, S.G., Brogden, R.N. Alprazolam, a review of its pharmacodynamic properties and efficacy in the treatment of anxiety and depression. Drugs 27, 132-147, 1984.

2. Rickels, K. Recent advances in anxiolytic therapy. J. Clin. Psychiatry, 42, 40-43, 1981.

3. Feighner, J.P., Aden, G.C., Fabre, L.F. et al. Comparison of alprazolam, imipramine and placebo in the treatment of depression. J.Am.Med. Assoc.,249, 3057-3064, 1983.

4. Feighner, J.P. The new generation of antidepressants. J. Clin. Psychiatry, 44, 49-55, 1983.

5. Fabre, L.F., McLendon, D.M. A double-blind study comparing the efficacy and safety of alprazolam with imipramine and placebo in primary depression Curr. Ther. Res.,27, 474-482, 1980.

6. Gonzalez, E.R. Panic disorder may respond to new antidepressants. J. Am. Med. Assoc.,248,3077-3087, 1982.

7. McCormick, S.R., Nielsen, J., Jatlow, P. Quantification of alprazolam in serum or plasma by liquid chromatography. Clin. Chem.30(10),1652-1655, 1984.

8. Adams, W.J., Bombardt, P.A. Brewer, J.E. Normal-phase liquid chromatographic determination of alprazolam in human serum. Anal. Chem.,56, 1590-1594, 1984.

9. Greenblatt, D.J., Divoll, M., Moschitto, L.J., Shader, R.I. Electron-capture gas chromatographic analysis of the triazolobenzodiazepines alprazolam and triazolam. J. Chromatogr. (Biomedical Applications),225, 202-207, 1981.

10. Miller, R.L., DeVane, C.L., Alprazolam, a-hydroxy- and 4-hydroxyalprazolam analysis in plasma by high-performance liquid chromatography. J. Chromatogr. (Biomedical Applications)430, 180-186, 1988.

11. Adams, W.J., Specific and sensitive high performane liquid chromatographic determination of alprazolam or triazolam. Anal. Lett.,12(B6), 657-671, 1979.

12. Theis, D.L., Bowman, P.B., Development of a liquid chromatographic method for the determination of triazolo-benzodiazepines. J. Chromatogr.,268, 92-98, 1983.

13. Fraser, A.D., Urinary screening for alprazolam, triazolam and their metabolites with the EMIT(R) d.a.u.™ benzodiazepine metabolite assay. J. Anal. Toxicol.11(6) 263-266, 1987.

14. Fraser, A.D., Bryan, W. Evaluation of the Abbott ADx and TDx serum benzodiazepine immunoassays for analysis of alprazolam. J. Anal. Toxicol.,15(2), 63-65, 1991.

15. Cho, M.J., Scahill, T.A., Hester, J.B. Kinetics and equilibrium of the reversible alprazolam ring- opening reaction. J. Pharm. Sci.,72, 356-361, 1983.

16. Abernethy, D.R., Greenblatt, D.J., Divoll, M., Shader, R.I. Pharmacokinetics of alprazolam. J. Clin. Psychiatry,44, 45-47, 1983.

17. Greenblatt, D.J., Divoll, M., Abernethy, D.R., Shader, R.I. Clinical pharmacokinetics of alprazolam, a triazolo benzodiazepine. Chap. 26 in The Affective Disorders, JM Davis, JW Maas, Eds., American Psychiatric Press, Inc, Washington, DC, pp 367-378, 1983.

18. Eberts, F.S., Philopaulos, Y., Reineke, L.M., Vliek, R.W. Disposition of ^{14}C-alprazolam, a new anxiolytic antidepressant in man. Pharmacologist, 22, 279, 1980.

19. Edinboro, L.E., Backer, R.C. Preliminary report on the application of a high performance liquid chromatographic method for alprazolam in postmortem blood specimens. J. Anal. Toxicol., 9(5), 207-208, 1985.