Direct Injection High-Performance Liquid Chromatography of Tetrabenazine and Its Metabolite in Plasma of Humans and Rats

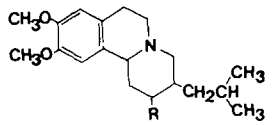
REZA MEHVAR*, FAKHREDDIN JAMALI*x, MICHAEL W. B. WATSON‡, AND DAVID SKELTON‡

Received July 11, 1985, from the *Faculty of Pharmacy and Pharmaceutical Sciences and the ‡Department of Geriatric Medicine, The University of Alberta, Edmonton, Alberta, Canada, T6G 2N8. Accepted for publication August 11, 1986.

Abstract □ A convenient, selective, and sensitive reversed-phase HPLC assay was developed to measure concentrations of the dopamine-depleting agent, tetrabenazine (1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-benzo(*a*)quinolizin-2-one) and its dihydro metabolite in the plasma of patients with tardive dyskinesia receiving therapeutic doses of the drug and in the plasma of rats. The method involves plasma protein precipitation, oxidation of the compounds with mercuric acetate at 110 °C for 1 h, addition of internal standard, and injection into the instrument. Fluorescence detection was utilized at excitation and emission wavelengths of 265 and 418 nm, respectively. The peaks from the drug, its metabolite, and at least three other substances were best resolved at 60 °C using a mobile phase of water:acetonitrile:acetic acid: triethylamine (65:33:2:0.15) at a flow rate of 0.6 mL/min; the 4.6 mm × 10 cm column contained 5 μm of octadecylsilane packing. To assess the applicability of the assay, the drug was administered intravenously to rats, and plasma concentrations were determined before (by UV-HPLC) and after (by fluorescence-HPLC) the oxidative procedure. In addition, the MS spectra of tetrabenazine and the dihydro metabolite, isolated from biological samples, were identical to those of authentic samples. Excellent linearity was observed between the peak area ratios and concentrations over the ranges 0.5–200 and 2–1000 ng/mL of the drug and the metabolite, respectively. Minimum quantifiable concentrations of the drug and its metabolite were 0.5 and 2.0 ng/mL, respectively. The sensitivity was found to be adequate for pharmacokinetic studies of tetrabenazine in humans and rats.

Tetrabenazine (1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-benzo(*a*)quinolizin-2-one; 1) is a synthetic benzoquinolizine derivative which, like reserpine, depletes monoamines in the central nervous system.[1] Originally introduced as an antipsychotic agent in 1958,[2] the drug failed to elicit potent antipsychotic properties and was consequently withdrawn from the market. Recently, however, its effect in controlling some movement disorders has attracted considerable attention.[3–6] It appears to be a drug of choice in the treatment of tardive dyskinesia (TD)[5] and Huntington's chorea.[6] However, treatment with 1 is empirical as the pharmacokinetics of this drug are unknown. This is mainly due to the unavailability of a suitable assay to determine the drug and its metabolites in biological samples. A previously reported HPLC method[7] involved a lengthy extraction procedure and, since an internal standard (IS) was not used, it appeared to be subject to considerable experimental error.

The objective of this paper is to report a sensitive, selective, and convenient HPLC assay of 1 and one of its major metabolites, the dihydro metabolite, 2, in the plasma of humans and rats.



1 R, =O
2 R, -OH

Experimental Section

Materials—Tetrabenazine (lot 61–84) and the dihydro metabolite (lot 39–85) were gifts from Hoffmann-LaRoche of Canada (Etobicoke, Ont.). Internal standards (IS) were 5,6-benzoquinoline (B.D.H. Laboratory, England) and diazepam (Sigma Chem. Co., St. Louis, MO). Other compounds investigated for their appropriateness as internal standards for the fluorescence-HPLC assay were: berberine HCl; coralyne chloride; corynanthine HCl; emetine HCl; fluorene (Aldrich, Milwaukee, WI); quinaldic acid; quinidine; reserpine; and yohimbine (Sigma Chem. Co., St. Louis, MO). Acetonitrile and water were HPLC grade, while triethylamine (TEA), acetic acid, ethyl acetate, sodium acetate, and mercuric acetate were analytical reagent grade.

Apparatus—A 590 solvent pump was attached to a Wisp 710-B autosampler (Waters, Mississauga, Ont.) and a GM 970 fluorometer (Kratos, Westwood, NJ). The detector was set at excitation and emission wavelengths of 265 and 418 nm, respectively. The chromatograms were recorded on a 3390A integrator (Hewlett-Packard, Mississauga, Ont.). The UV absorbance of 1 and 2 were measured using a variable-wavelength detector (model 481, Waters, Mississauga, Ont.) at 280 nm.

Mass spectra (electron impact and chemical ionization) were obtained on a Varian 6000 GL/VG 7070E MS/POP 11/24 data system (AnalyTech Instrumentation and Service, St. Laurent, Canada). For chemical ionization (CI) spectra, ammonia was used as the reactant gas.

Standard Solution—For the fluorescence method, a 3% (w/v) solution of mercuric acetate in water containing 5% acetic acid and 5% sodium acetate was prepared. Stock solutions of 1 and 2 were prepared by dissolving the compounds in ~2 mL of methanol and then adding water to a final volume of 100 mL. Blank plasma was spiked with standard solutions of the compounds prepared by diluting the stock solution to contain final concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL for tetrabenzine (1) and the dihydro metabolite 2, and 500 and 1000 ng/mL for 2. To examine the effect of plasma on the fluorescence intensity of the compounds, similar solutions with final concentrations of 0.5, 1, 5, 20, and 100 ng/mL of 1, and 5, 20, and 100 ng/mL of 2 were prepared in duplicate in the absence of plasma. The internal standard solution contained 50 ng/mL of 5,6-benzoquinoline.

For UV detection, stock solutions of 1 and 2 were prepared in 5 mM hydrochloric acid. Blank plasma was spiked with the stock solution to produce final concentrations of 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L of the drug and its metabolite. The internal standard solution was prepared by dissolving 10 mg of diazepam in 200 mL of methanol.

Calibration curves were constructed by plotting the peak area ratio of the compounds to IS versus the plasma concentrations of 1 and 2.

Sample Preparation—For the fluorescence assay, 0.4 mL of the mercuric acetate solution was combined with 0.4 mL of plasma in a microcentrifuge tube. The tubes were vortexed for 30 s, centrifuged (microcentrifuge model 235A, Fisher, Edmonton, AB) for 5 min, and 0.6 mL of the supernatant was transferred to glass tubes. The tubes were then heated at 110 °C for 1 h. After cooling to ambient temperature, 0.1 mL of the internal standard solution was added, and the contents of the tubes were transferred to autosampler vials. Depending on the concentration, aliquots of 25 to 100 μL were then injected into the instrument.

For the UV assay, 1 mL of plasma was combined with 50 μL of the internal standard, 0.1 mL of 0.2 M NaOH and 4 mL of ethyl acetate.

0022-3549/86/1000-1006$01.00/0

The tubes were vortex mixed for 30 s and centrifuged (Adams Dynac, USA high-speed centrifuge) for 3 min at 5000 rpm. The organic layer was evaporated using a concentrator/evaporator (Savant Speed Vac; Emerston Instrument, Scarborough, Canada), and the residue was dissolved in 0.2 mL of acetonitrile. Aliquots of 10 to 25 μL of the reconstituted solutions were injected into the HPLC. To estimate the extraction efficacy of this method, blank plasma samples were spiked with internal standard and extracted as described above. After evaporation, the contents of the tubes were dissolved in 0.2 mL of acetonitrile containing tetrabenazine and its metabolite to give final concentrations corresponding to those of 1 and 5 mg/L in plasma (n = 2). Aliquots of 0.02 mL of the solutions were then injected into the HPLC apparatus, and the responses were compared with those observed following extraction from solutions of equal concentrations.

Chromatography—Resolution of 1 and 2, their dehydro derivatives, and at least three unknown metabolites, was achieved using a 4.6 mm × 10 cm analytical column containing 5 μm of octadecylsilane packing material (Partisil 5 ODS-3; Whatman, Clifton, NJ) at 60 °C (model 1122/WTC-120 oven, Waters, Mississauga, Ont.) for the fluorescence-HPLC analysis, and at ambient temperature for the UV-HPLC analysis with a precolumn module (Guard-Pak with Bondapak C18 end-capped inserts; Waters, Mississauga, Ont.). Other reversed-phase columns tested for their performance were 3.9 mm × 30 cm stainless steel μBondapak C18, 8 mm × 10 cm Novapak C18 Radial-Pak, and 8 mm × 10 cm μBondapak C18 Radial-Pak from Waters (Mississauga, Ont.); 4.16 mm × 12.5 cm Partisphere cartridge (Whatman, Clifton, NJ); and 4.6 mm × 25 cm stainless steel RP-18 (Serva, Heidelberg, FRG).

For the fluorescence assay, the mobile phase consisted of water: acetonitrile:acetic acid:triethylamine (65:33:2:0.15). The flow rate was set at 0.6 mL/min. The UV analysis was performed using a solution of water:acetonitrile:triethylamine:acetic acid (50:50: 0.2:0.1) as the mobile phase. The flow rate and column were the same as those described for the fluorescence method. The pH of the aqueous component of the mobile phases were 3.8 and 6.2 for the fluorescence and UV assays, respectively.

Patient Study—Two patients affected by tardive dyskinesia (TD), whose movements were controlled by **1**, and a healthy subject volunteered to participate in the project. The objectives and implications of the experiment were explained to them and signed, informed consent forms were obtained. Patient 1 was a 42 year old male weighing 120 kg who was taking 25 mg of the drug tid. He was also receiving chlorpromazine (100 mg, tid) and diazepam (5 mg, tid), both at 6:00, 14:00, and 20:00 h. Patient 2 was a 62 year old female weighing 75 kg who was under treatment with 37.5 mg of tetrabenazine tid. She was also taking acetaminophen (650 mg, qid). Both patients appeared to have normal kidney and liver functions. The drug (Nitoman tablets, Hoffmann-LaRoche, Etobicoke, Ont.) was administered at 8:00, 13:00, and 18:00 h. Blood samples (2 mL) were taken just before and then 0.5, 1.0, 2.0, 3.0, and 5.0 h after the first and second dose, and 0.5, 1.0, 2.0, 3.0, and 14.0 h after the last dose. The healthy volunteer was a 70-kg, 39-year-old male who took a single oral dose of 25 mg of the drug and donated blood samples just before and 1.5 h after the dose. He did not take any other medications 2 weeks before and during the experiment. Blood samples were collected in Vacutainer tubes (Becton Dickinson, Mississauga, Ont.) containing potassium oxalate and sodium fluoride as anticoagulants. Plasma was separated and kept frozen at −20 °C until analyzed.

Animal Study—Tetrabenazine (1 mg/kg dissolved in 1 mM HCl) was injected into the jugular vein of four male Sprague-Dawley rats, weighing 250–300 g, through a previously inserted catheter. Blood samples (0.25 mL) were taken at 0, 5, and 30 min and at 2, 4, 8, 12, and 23 h and transferred into microcentrifuge tubes containing sodium citrate. Blood plasma was separated and assayed for 1 and 2 as described above.

Validation Test—Aliquots of the plasma samples from patient 2 were pooled (total of 3 mL) and assayed for 1 and 2 before (UV detection) and after (fluorescence detection) oxidation.

Under light anesthesia, doses of the drug (1–5 mg/kg) were injected through the jugular vein into five Sprague-Dawley male rats weighing 250–300 g. Approximately 10 min after administration, 3–10 mL of blood were collected in the presence of sodium citrate as anticoagulant. Plasma was separated and kept at −20 °C until analysis. The specimens were assayed using both UV detection for unchanged 1 and 2, and fluorescence detection for oxidized 1 and 2. The estimated concentrations were then compared.

The identities of 1 and 2 in the biological samples were confirmed by comparing the electron impact and chemical ionization GC-MS properties of the peaks separated from UV-HPLC eluants with those of authentic standards.

Results and Discussion

An advantage of reversed-phase chromatography is that the biological samples can be directly injected into the HPLC provided that the detector possesses sufficient sensitivity. We have previously reported application of the direct-injection method in analyzing nonsteroidal anti-inflammatory drugs (NSAID).[8] The therapeutic plasma concentrations of NSAIDs are usually in the range of μg/mL, thus their quantification is conveniently achieved using ultraviolet detectors. The plasma concentrations of 1 following administration of the recommended doses, on the other hand, seem to be in the range of ng/mL, which is beyond the normal range of existing UV detectors. Fluorescence detection seems to be an appropriate alternative. However, neither the drug nor its known metabolite possesses strong fluorogenic properties. Schwartz and co-workers[9,10] used TLC to separate 1 and its metabolites. They then visualized the resolved compounds by spraying them with a solution of mercuric acetate and heating the plates at 110 °C for 10 min. Under these conditions, benzoquinolizine derivatives are expected to be oxidized to highly fluorescent derivatives. Leonard et al.,[11] who used mercuric acetate to dehydrogenate the bicyclic tertiary amine, quinolizidine, identified the product as $\Delta^{1(10)}$-dehydroquinolizidine. A similar dehydrogenated product for tetrabenazine, a tricyclic tertiary amine, is feasible. Roberts et al.[7] utilized this reaction for the HPLC analysis of 1 and 2 in plasma extracts. However, their method[7] had two main shortcomings: it involved a lengthy extraction procedure and was carried out in the absence of an internal standard. According to Roberts et al.,[7] this method could measure ~0.1 ng/mL of 1 and 1.0 ng/mL of 2 in plasma; in our hands, 1 and 2 could not be determined when their respective concentrations were <10 and 50 ng/mL. However, when the extraction process was eliminated from the sample preparation procedure, the detection limit was improved by several fold. It is, therefore, reasonable to suggest that the extraction method used by Roberts et al.[7] may have a poor efficiency. Using the direct-injection method reported here, the minimum quantifiable concentrations for 1 and 2 were found to be 0.5 and 2.0 ng/mL, respectively. The signal:noise ratios for 0.5 and 2.5 ng/mL of 1 and 2 in plasma preparations were >6 and >8, respectively. Concentrations of <0.5 and <2.0 ng/mL of 1 and 2, respectively, however, were associated with coefficients of variation of >10%.

Various reagents including acetonitrile, trichloroacetic acid, and zinc sulfate were used to precipitate plasma proteins. The most satisfactory results were obtained following the addition of mercuric acetate, which is also required for the oxidation of 1 and 2. At least 400 injections were made into the same column before a significant reduction in its efficiency was noticed.

To determine the time required to complete the dehydrogenation, 1 and 2 were heated in the presence of mercuric acetate for 2 h at 110 °C. The fluorescence intensity of the solutions were measured at 15-min intervals. At 110 °C, the optimum time was found to be 45 min for 1 and 1 h for 2. Since the intensity remained constant after 1 h, this heating time was chosen for the purpose of oxidizing 1 and 2.

Figure 1 depicts chromatograms of plasma samples from a healthy subject before, and 1.5 h after, administration of 25 mg of 1, and from patient 2 just prior to the first routine daily dose. Retention times for 1 and 2 were 7.5 and 5.5 min, respectively. For the healthy subject, three additional plasma components, presumably other metabolites of 1, were observed at 3.1, 3.6, and 4.4 min. In their HPLC trace,

Figure 1—*Chromatograms of plasma* (a) *from a healthy subject before and* (b) *1.5 h after the administration of 25 mg of tetrabenazine and* (c) *from a patient taking 37.5 mg of tetrabenazine tid for 8 months (sample taken just before the first daily dose). Key:* 1, 2, *and* 3 *are tetrabenazine, its dihydro metabolite, and internal standard (5,6-benzoquinoline), respectively. At a detector sensitivity of 1.0, the recorder attenuation was maintained at 2, except from 7 to 9 min when it was changed to 0.*

Roberts et al.[7] detected only one peak in addition to 1 and 2, and suggested that it might represent co-eluting metabolites of 1. This can be attributed to the deficiency of the column used by these authors, as we also noticed that with the exception of Partisil 5 ODS-3, all of the tested columns exhibited poor peak resolution properties.

Plasma of the patients contained an extra peak in addition to the ones found in the specimen collected from the healthy subject. This peak eluted 2.6 min after injection. The extra peak can be attributed either to the accumulation of a metabolite or a group of metabolites eluted together, when 1 is given repeatedly, or to the presence of other drugs concomitantly taken by the patients. However, as the nature of these additional drugs, i.e., acetaminophen in one case and diazepam and chlorpromazine in another, are so different from one another, it is unlikely that they appear in plasma as fluorogenic compounds with the same retention time. The extra peak, therefore, appears to result from the administration of 1.

Figure 2 depicts chromatograms of rat plasma before, and

Figure 2—*Typical chromatograms of rat plasma using HPLC with fluorescence detection* (a *and* b) *and HPLC with UV detection* (c *and* d) *before* (a *and* c) *and 2 h* (b) *and 10 min* (d) *after 1 mg/kg and 5 mg/kg iv doses of the drug, respectively. Key:* 1 *and* 2 *represent tetrabenazine and the dihydro metabolite;* 3 *(5,6-benzoquinoline) and* 4 *(diazepam) are internal standard peaks.*

10 min after, intravenous administration of 1 mg/kg of tetrabenazine. The chromatograms of plasma samples from humans and rats are similar with respect to the number and retention times of observed peaks.

Reversed-phase HPLC of nitrogen-containing compounds may result in broad asymmetric peaks.[12,14] This has been attributed to the adsorption of these compounds to the free residual silanol groups on the silica gel column packing. Peaks representing dehydrogenated 1 and 2 were broad and showed considerable tailing in the absence of triethylamine. Furthermore, without triethylamine, the relationship between the peak area ratios and the corresponding concentrations was nonlinear for both 1 and 2, indicating considerable adsorption to the column. The nonlinearity was particularly evident when Novapak (Radial-Pak) columns were used. The extent of adsorption was greater for 2 than for 1. Adsorption problems were eliminated by addition of triethylamine and increasing the column temperature to 60 °C. Among the compounds examined for their suitability as internal standards, the aromatic compound, 5,6-benzoquinoline, was selected because it showed favorable fluorogenic properties and was completely resolved from other peaks.

Excellent linearity was found between the peak area ratios (1/IS and 2/IS) where IS is the internal standard, and plasma concentrations within the ranges of 0.5–200 and 2–1000 ng/mL for 1 and 2, respectively. The best-fit lines passing through the experimental points were $y = 0.0013 + 0.0392x$ and $y = 0.0038 + 0.0053x$, with correlation coefficients of 0.999 and 0.998 for 1 and 2, respectively. Within the examined range, the intraday CVs were within the range of 1.9 to 6.3% for 1 and 2.2 to 6.5% for 2. The interday CVs were 2.8 to 7.9% and 2.6 to 8.0% for 1 and 2, respectively. The differences between the concentrations added to plasma and those found ranged from 0.1 to 9.9% within the examined range (Table I). The recovery of the compounds from spiked plasma samples was ~100% (94.3–104.6% for 1 and 97.4–103.6% for 2).

To examine if the oxidation products were indeed derived only from the drug and its metabolite, we developed a UV-HPLC method which enabled us to measure 1 and 2 in plasma samples at concentrations as low as 0.5 μg/mL (Fig. 2). Within the range of 0.5–10 μg/mL, the relationship between the concentrations and peak area (1:IS or 2:IS) was linear (coefficient of correlation >0.991), with acceptable reproducibility (CV <8%, n = 3). The efficacy of the extraction method was between 92.0–97.0% and 100.0–101.2% for 1 and 2, respectively. Plasma concentrations of the drug and its metabolite, following intravenous administration of 1–5 mg/kg to rats, were then measured using both UV- and

Table I—Accuracy and Precision of the Fluorescence–High-Performance Liquid Chromatographic Assay Performed on Spiked Plasma Samples[a]

Theoretical Conc., ng/mL	Conc. Found, ng/mL			
	Tetrabenazine (1)	% Error	Dihydro Metabolite (2)	% Error
0.5	0.49 (7.9)	−2.0	—	—
1.0	1.08 (4.9)	8.0	—	—
2.0	2.17 (7.8)	8.5	1.88 (5.1)	−6.0
5.0	4.97 (4.9)	−6.0	4.78 (6.7)	−4.4
10.0	9.99 (3.9)	−0.1	9.01 (8.0)	−9.9
20.0	19.66 (2.8)	−1.7	18.82 (3.8)	−5.9
50.0	48.14 (5.2)	−3.7	51.54 (2.6)	3.1
100.0	102.42 (5.6)	2.4	104.51 (3.6)	4.5
200.0	199.38 (2.9)	−0.3	196.5 (4.8)	−1.8
500.0	—	—	510.6 (3.1)	2.1
1000.0	—	—	1001.2 (2.5)	0.1

[a] Values in parentheses are % CV; n = 4.

fluorescence-HPLC (Table II). The observed differences and disproportionalities in the plasma concentrations reported in Table II cannot be attributed only to the interanimal variations. As blood samples were not collected at precisely the same time and the drug concentration declined very rapidly during the first few minutes postdosing (Fig. 3), the variations might, at least partly, be attributed to the different sampling time. The values estimated before (UV) and after (fluorescence) oxidation were similar (two-way ANOVA at $\alpha = 0.05$). This indicates that, indeed, the oxidized derivatives are derived only from 1 and 2. As the therapeutic plasma concentration of the drug is below the minimum quantifiable concentration for UV-HPLC, plasma samples from patient 2 were pooled and extracted with ethyl acetate. This procedure enabled us to compare the plasma concentration of 2 using both methods of detection. The values were 0.609 and 0.598 μg/mL using UV- and fluorescence-HPLC, respectively.

The electron impact GC-MS properties of 1 and 2, collected from UV-HPLC eluants of plasma samples, were identical to those of the respective authentic standards. The major fragments, *m/z* (% relative abundance), for 1 included: 317 (M^+, 16), 316(18), 274(40), 261(78), 260(33), 232(18), 192(25), 191(100), 190(32), 176(35); and for 2 included: 319 (M^+, 55), 318(48), 274(53), 232(36), 206(28), 205(100), 192(27), 191(98), 190(24), 176(24). The CI-mass spectra indicated quasi-molecular ions of *m/z* and 320 for 1 and 2, respectively.

Table II—Rat Plasma Concentrations of Tetrabenazine and the Dihydro Metabolite[a]

Rat	Dose, mg/kg	Tetrabenazine (1)		Dihydro Metabolite (2)	
		UV	Fluorescence	UV	Fluorescence
1	1.0	0.288	0.279	0.506	0.431
2	5.0	5.316	5.004	0.585	0.606
3	3.0	3.063	2.826	1.134	1.328
4	1.0	0.777	0.895	0.981	0.934
5	1.0	1.068	1.021	1.038	1.141

[a] Measured by UV- and fluorescence–HPLC ~10 min after intravenous administration of the drug.

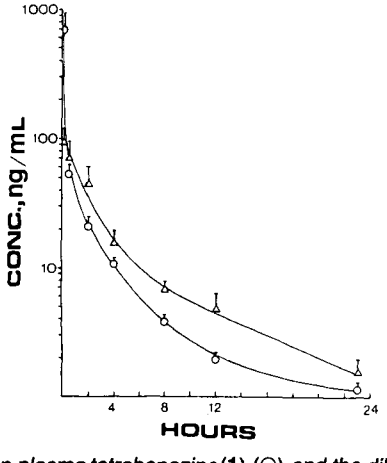


Figure 3—*Mean plasma tetrabenazine* (1) (○) *and the dihydro metabolite* 2 (△) *concentration–time curves following iv administration of 1 mg/kg of the drug to four rats. Bars represent standard error from the mean.*

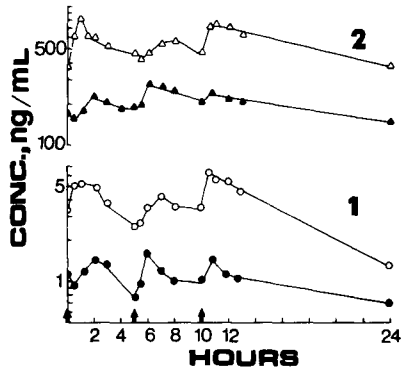


Figure 4—*Steady-state time course of tetrabenazine* (1) *and its dihydro metabolite* 2 *in patients receiving 25 mg* (●, ▲) *and 37.5 mg* (○, △) *of* 1 *tid, respectively. Arrows indicate times of administration.*

Figure 4 depicts the time course of 1 and 2 in the plasma of two patients following oral administration of 1. For the unidentified compounds, only the peak area ratio (compound: internal standard) could be plotted versus time. The time course of 1 and 2 following intravenous administration of 1 to rats is shown in Fig. 3.

This method can be utilized to monitor plasma concentrations and delineate pharmacokinetics of tetrabenazine and its dihydro metabolite in rats and also in patients receiving the recommended dosage of the drug.

References and Notes

1. Pletscher, A.; Brossi, A.; Gey, K. F. *Neurology* **1962**, *4*, 275–306.
2. Brossi, A.; Lindlar, H.; Walter, M.; Schnider, O. *Helv. Chim. Acta* **1958**, *41*, 119–139.
3. Swash, Michael; Robert, A. H.; Zakko, Hazim; Healthfield, K. W. G. *J. Neurol. Neurosurg. Psychiatry* **1972**, *35*, 186–191.
4. Jankovic, Joseph *Ann. Neurol.* **1982**, *11*, 41–47.
5. Fahn, Stanley *Adv. Neurol.* **1983**, *37*, 267–276.
6. McLellan, D. L.; Chalmers, R. J.; Johnson, R. H. *Lancet* **1974**, *1*, 104–107.
7. Roberts, M. S.; Watson, H. M.; McLean, S.; Millingen, K. S. *J. Chromatogr.* **1981**, *226*, 175–182.
8. Jamali, F.; Russell, A. S.; Berry, B. W.; Lehmann, C. *J. Chromatogr.* **1984**, *310*, 327–333.
9. Schwartz, D. E.; Rieder, J. *Clin. Chim. Acta* **1961**, *6*, 453–463.
10. Schwartz, D. E.; Bruderer, H.; Rider, J.; Brossi, A. *Biochem. Pharmacol.* **1966**, *15*, 645–655.
11. Leonard, Nelson J.; Hay, Allan S.; Fulmer, Richard W.; Gash, Virgil W. *J. Am. Chem. Soc.* **1955**, *77*, 439–444.
12. DeSilva, J. A. F.; Strojny, N.; Munno, N. *J. Pharm. Sci.* **1973**, *62*, 1066–1074.
13. Sokolowski, A.; Wahlund, K. G. *J. Chromatogr.* **1980**, *189*, 299–316.
14. Weber, Stephen G.; Tramposch, G. *Anal. Chem.* **1983**, *55*, 1771–1775.

Acknowledgments

This research was supported in part by Hoffmann-LaRoche of Canada. The helpful assistance of Mrs. Jane Cowley, research nurse, is appreciated. The authors are grateful to Dr. F. M. Pasutto of the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, for interpretation of mass spectra. Part of this work was presented at the 45th International Congress of Pharmaceutical Sciences, Montreal, P.Q., Canada, September, 1985.