Journal of Chromatography, 493 (1989) 392–397
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4831

Note

Mass spectrometric determination of tetrabenazine using a stable isotope-labeled analogue as an internal standard

S.P. JINDAL*, T. LUTZ and S.P. BAGCHI

The Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962 (U.S.A.)

(First received February 9th, 1989; revised manuscript received April 21st, 1989)

Tetrabenazine (Nitoman) is a synthetic benzoquinolizine derivative (1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzoquinolizine-2-one) and, like reserpine, depletes brain monoamines [1,2]. This drug has a definite antipsychotic action [3] and has been found useful in treating Huntington's chorea [4] and tardive dyskinesia [5]. Tetrabenazine action upon dopamine, among various brain monoamines, correlates strongly with its clinical effects. The release of dopamine from synaptic vesicles is a significant action of this drug [6] and, based on its interaction with cocaine and phencyclidine (PCP) at the vesicular level, tetrabenazine has also been suggested for controlling cocaine and PCP abuse effects [7,8].

Any clinical application of tetrabenazine has been hampered due to the unavailability of a suitable assay to determine the drug and its metabolites in biological samples. Recently an assay of tetrabenazine in human plasma, based on high-performance liquid chromatographic (HPLC) methodology, appeared [9,10]. This assay utilized no appropriate internal standard and therefore could not be precise and specific. This report describes a sensitive and selective gas chromatographic–mass spectrometric (GC–MS) assay of tetrabenazine in rat brain and plasma employing selected-ion monitoring (SIM) [11,12] and deuterium-labeled tetrabenazine as an internal standard.

EXPERIMENTAL

Tetrabenazine was a gift from Hoffman-La Roche (Nutley, NJ, U.S.A.). ^2H_2O, 99.8 atom-% deuterium, pentafluoropropionic anhydride, trifluoroacetic

0378-4347/89/$03.50

anhydride and diethylamine (Aldrich, Milwaukee, WI, U.S.A.) were used without further purification. All solvents were of analytical grade (Fisher Scientific, Pittsburgh, PA, U.S.A.); silanized tubes (10 ml) with screw caps were used for extraction and final solvent evaporation was performed in 5-ml glass stoppered centrifuge tubes (Kimble Owens, Toledo, OH, U.S.A.). Pasteur pipettes with hand-drawn constricted tips were utilized for all solvent transfers.

[$^2H_2$]Tetrabenazine

Tetrabenazine (5 mg) in 10 ml of anhydrous tetrahydrofuran and 1 ml of pentafluoropropionic [^{2}H]acid (prepared in situ from a 1:1 molar mixture of pentafluoropropionic anhydride and ^2H_2O) was heated under reflux for 3 h. After this period, the material was cooled to room temperature, adjusted to pH 9.0 and extracted with benzene. Enolic H atoms undergo a typical acid-catalyzed exchange process [13,14] resulting in the formation of [$^2H_2$]tetrabenazine. The product was chromatographically identical to the authentic unlabeled material. A selected-ion detection analysis of the material showed the presence of an ion equivalent to $96 \pm 0.5\%$ [$^2H_2$]tetrabenazine.

Instrumentation

Preliminary GC was performed on a Perkin-Elmer Model 3920 instrument (Norwalk, CT, U.S.A.), equipped with a silanized 1.8-m column packed with 1% OV-1 on Gas Chrom Q (100–200 mesh) and maintained at 200°C with a detector temperature of 275°C. The carrier gas flow-rate was 30 ml/min. GC–MS work was carried out on an LKB-9000 instrument (Stockholm, Sweden) equipped with a multiple-ion detector–peak matcher (MID–PM) accessory [11,12]. The GC column temperature was 200°C, the flash heater at 220°C, the ion source at 250°C, and the helium flow-rate was 20 ml/min. The retention time of tetrabenazine was 3.5 min while that of tetrabenazine enol trifluoroacetate was 2 min. For optimal noise-to-signal ratio, the ionization potential was 70 eV in the scan mode, 20 eV in the SIM mode, and the trap current was set at 60 μA.

Extraction and derivatization of tetrabenazine

Drug-free plasma (1 ml) or rat brain extract in 0.1 *M* hydrochloric acid (1.0 ml) was spiked with 22.5 ng of tetrabenazine, and to the samples were added 25 μl of a stock solution of [$^2H_2$]tetrabenazine (concentration of stock solution 0.8 ng/μl). Following the addition of 3.0 ml of water, 1.0 ml of borate buffer, pH 9.0, the samples were thoroughly mixed on a vortex mixer, adjusted to pH 8.8 with 1 *M* ammonium hydroxide and extracted with 10 ml of benzene. From each sample, the organic layer was separated and to it 1 ml of 0.1 *M* hydrochloric acid was added; the mixture was shaken for 15 min. The organic

layer was discarded and the aqueous phase was mixed with 1.0 ml of borate buffer and extracted with 5.0 ml of benzene. The organic layer was dried with sodium sulphate and evaporated to dryness under a gentle stream of nitrogen at 50°C. To the dried residue were added 100 μl of a diethylamine–benzene solution (2%, v/v) and 100 μl of trifluoroacetic anhydride, and the material was heated at 90°C for 1 h. As expected under these conditions tetrabenazine is converted quantitatively to tetrabenazine enol trifluoroacetate. After this reaction, the material was evaporated to dryness under a gentle stream of nitrogen at room temperature and dissolved in 50 μl of benzene; 1.5 μl were injected into the GC–MS system and appropriate ion intensities were measured.

RESULTS AND DISCUSSION

The mass spectrum of tetrabenazine shows a molecular ion at *m/z* 317, a fairly significant ion at *m/z* 316 due to a loss of a H atom, a peak of modest

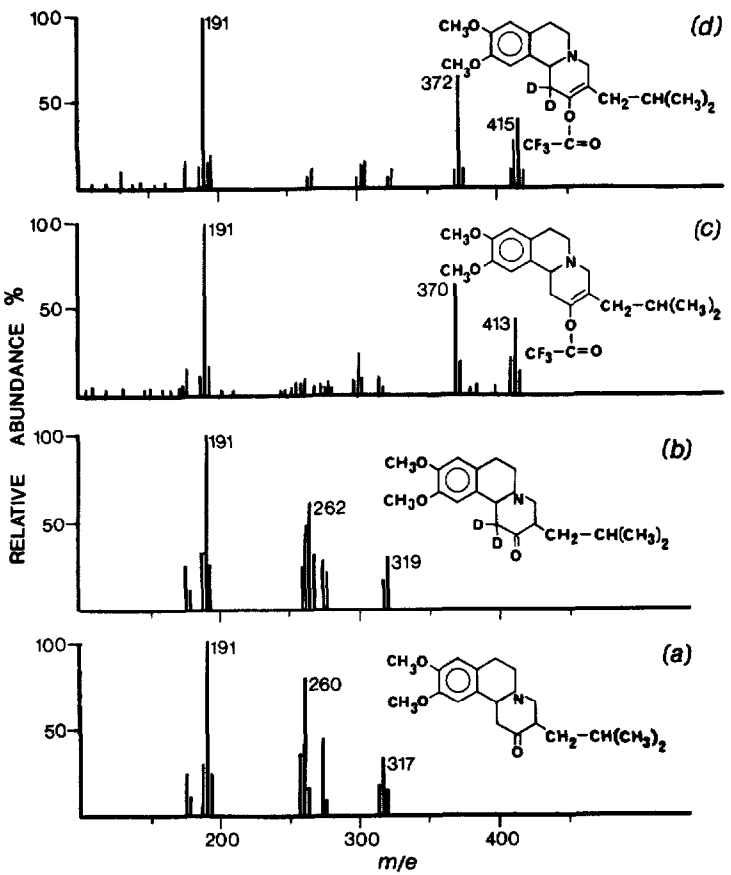

Fig. 1. Electron-impact mass spectra of (a) tetrabenazine, (b) [$^2H_2$]tetrabenazine, (c) tetrabenazine enol trifluoroacetate and (d) [$^2H_2$]tetrabenazine enol trifluoroacetate.

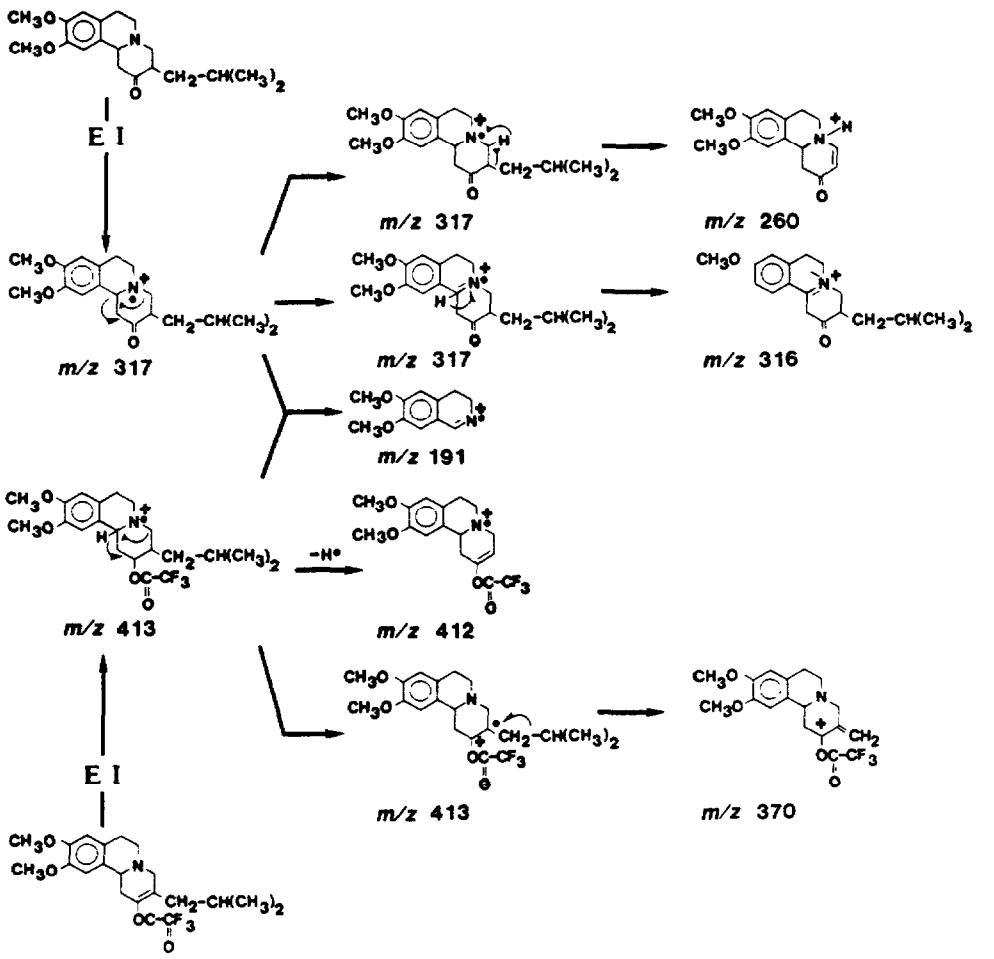

Fig. 2. Proposed electron-impact (EI) fragmentation mechanism.

intensity at *m/z* 260 again with a relatively intense ion at *m/z* 259 and a base peak at *m/z* 191. The [$^2H_2$]tetrabenazine spectrum shows similar characteristics with appropriate ions shifted to higher mass by 2 a.m.u. while the base ion, being the common ion between the labeled and the unlabeled tetrabenazine, appears at *m/z* 191 (Fig. 1). The fragmentation mechanism is readily discernible (Fig. 2). The molecular ion formed by loss of a lone pair electron from a nitrogen atom undergoes typical β-cleavage fragmentation processes to give an odd electron ion at *m/z* 191 and even electron ion at *m/z* 316 (318 in the case of [$^2H_2$]tetrabenazine). Obviously tetrabenazine is thermally stable and is suitable for GC–MS analysis; however, because of the presence of significant cluster of ions at *m/z* 260–259 and 317–316 SIM analysis lacked sensitivity as well as specificity. Consequently, tetrabenazine was converted to the

corresponding trifluoroacetate. Tetrabenazine trifluoroacetate, on GC–MS analysis, shows a molecular ion at *m/z* 413, a base peak at *m/z* 191 and an intense peak at *m/z* 370. The even electron ion at *m/z* 370 lacks any observable cluster of ions and is quite suitable for SIM analysis. The mass spectrum of the [$^2H_2$]tetrabenazine derivative is similar to that of the unlabeled isomer except that the appropriate ions are shifted to higher mass by 2 a.m.u.

Selected-ion monitoring assay

The ion at *m/z* 370 is selective for the tetrabenazine derivative (*m/z* 372 for the isotopomer) and is also a convenient working mass for the SIM assay. Furthermore, control plasma or tissue extract, subjected to the described procedure for tetrabenazine analysis, showed no significant background ions at *m/z* 370 and 372. Consequently, biological extracts, along with the labeled tetrabenazine, were processed as described above. An aliquot of the material was injected into the gas chromatograph–mass spectrometer, tetrabenazine was quantitated by measuring ion intensities at *m/z* 370 and 372, respectively, and standard curves were established in the usual manner. These data affirm a simple linear relationship between the appropriate ion intensity ratios and concentration of tetrabenazine and exclude any isotopic exchange or any significant kinetic isotope effect in the fragmentation process.

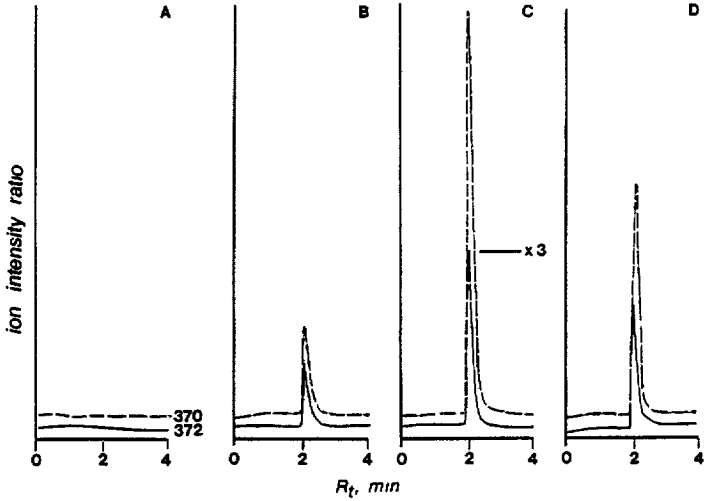

Fig. 3. Selected-ion fragmentograms of tetrabenazine enol trifluoroacetate (*m/z* 370) (broken line) along with [$^2H_2$]tetrabenazine enol trifluoroacetate (*m/z* 372) (solid line). (A) Drug-free control plasma extract (1 ml). (B) Plasma extract containing 26 ng/ml tetrabenazine and 20 ng/ml [$^2H_2$]tetrabenazine. (C) Extract from brain homogenate with added [$^2H_2$]tetrabenazine (17 ng/ml). Rats were given tetrabenazine (5 mg/kg) in isotonic saline intraperitoneally. After 10 min the animals were decapitated, the brain was removed and homogenized in 1 ml of ice cold 0.1 *M* hydrochloric acid and processed as described; tetrabenazine found was 800 ng in whole brain. (D) Extract from brain homogenate. In this case the animals were sacrificed 20 min after administration of dose and tetrabenazine found was 235 ng in whole brain.

Recovery and precision

Five samples containing 26 ng of tetrabenazine were analyzed as above using 20 ng/ml [$^2H_2$]tetrabenazine as an internal standard. The results of these samples were 24.5 ± 0.5 ng/ml. These samples were analyzed in duplicate; in the second set exactly the same amounts were taken as above but the internal standard was added after extraction. The recoveries for these samples, based on comparison of the ion intensity ratios of the two sets, were $82 \pm 8\%$. The wide range of recoveries observed is expected in trace analysis and is attributed to variable glassware, GC column absorption and possible thermal decomposition. The limit of determination of the assay, being a function of extraction efficiencies, GC column conditions and ion source, cannot be quoted in absolute terms. With good MS performance, a clean and freshly silanized GC column and 70% recoveries, a limit of determination of approximately 1–2 ng of tetrabenazine per ml is possible.

The methodology described above was used for the analysis of free drug in the whole brain of rats given 5.0 mg/kg tetrabenazine intraperitoneally and decapitated after a specific time period. The analyses of a typical extract of whole brain tissue showed the tissue content of the drug to be 800 ng, indicating extensive metabolism of the native drug. Mass chromatograms obtained from biological extract (Fig. 3) are clean, the peaks are symmetrical, and obviously no interference from extraneous materials is indicated. The analytical methodology described here will be used in future biotransformation studies of this important psychoactive drug.

ACKNOWLEDGEMENT

This work was supported by the Office of Research of the Department of Mental Hygiene of State of New York.

REFERENCES

1 A. Pletscher, A. Brossi and K.F. Gey, Neurology, 4 (1962) 275.
2 D.J. Pettibone, J.A. Totaro and A.B. Pflueger, Eur. J. Pharmacol., 102 (1984) 425.
3 O. Ling Jaerde, Acta Psychiatr. Scand., Suppl., 170 (1963) 15.
4 D.L. McLellan, D.J. Chalmers and R.H. Johnson, Lancet, i (1974) 104.
5 S. Fahns, Adv. Neurol., 37 (1983) 267.
6 D. Scherman, G. Boschi, R. Rips and J.P. Henry, Brain Res., 370 (1986) 176.
7 S.P. Bagchi, Biochem. Pharmacol., 32 (1983) 2851.
8 S.P. Bagchi, Neuropharmacology, 24 (1985) 37.
9 M.S. Roberts, H.M. Watson, S. McLean and K.S. Millingen, J. Chromatogr., 226 (1981) 175.
10 R. Mehvar, F. Jamali, M.W.B. Watson and D.J. Skelton, Pharm. Sci., 75 (1986) 1006.
11 C.G. Hammar, B. Holmstedt and R. Ryhage, Anal. Biochem., 25 (1968) 532.
12 B. Holmstedt and L. Palmer, Adv. Biochem. Psychopharmacol., 7 (1973) 1.
13 V. Fell, J.A. Hoskins and R.J. Pollitt, Clin. Chem. Acta, 83 (1978) 259.
14 S.P. Bagchi, T. Lutz and S.P. Jindal, J. Chromatogr., 344 (1985) 362.