Quantitation of Etorphine in Urine by Selective Ion Monitoring Using Tritiated Etorphine as an Internal Standard

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Abstract □ Selective ion monitoring combined with GLC was used for the assay of etorphine in urine. Commercially available tritiated etorphine was added as an internal standard. The advantage of the methodology using this internal standard is higher sensitivity by a factor of about 20 when compared with ordinary GLC.

Keyphrases □ Etorphine—GLC-selective ion mass spectrometric analysis, urine □ GLC-selective ion mass spectrometry—analysis, etorphine in urine □ Narcotic analgesics—etorphine, GLC-selective ion mass spectrometric analysis in urine

Etorphine, 6,14-*endo*-etheno-7-[1-(*R*)-hydroxy-1-methylbutyl]-6,7,8,14-tetrahydroripavine, as synthesized by Bentley and Hardy (1), is an analgesic approximately 1000 times as potent as morphine (2, 3). The fact that it works sublingually and has abuse potential (4) raises the possibility that it may become a future threat in the drug abuse field. Its extreme potency makes it a valuable tool for the study of narcotic receptors (5) but makes it difficult to detect after administration of pharmacologically effective doses.

Etorphine could not be detected in the urine with commonly available methods, including GLC, after the administration of high euphoronigenic doses in humans (6). Therefore, a sensitive and specific analytical method for this compound is needed.

Selective ion monitoring, the technique built on combined GLC–mass spectrometry with selective focusing on suitable fragments of the molecular ion (mass fragmentography) or the molecular ion itself, is used widely in pharmacology and biology (7, 8). This technique was used to develop a very sensitive and specific method for the analysis of etorphine in rabbit urine with commercially available $^3H_2$-etorphine as an internal standard.

EXPERIMENTAL

Nonlabeled etorphine[1], 15,16(*n*)-$^3H_2$-etorphine (specific activity[2] 41 Ci/mmole), and *N,O*-bis(trimethylsilyl)trifluoroacetamide[3] were obtained commercially and were used as received.

All animals were male, New Zealand White rabbits.

Extraction of Etorphine from Urine—To 2 ml of fresh urine was added an appropriate amount (36,000 cpm) of tritiated etorphine as an internal standard. The urine was saturated with sodium chloride, adjusted to pH 9.0 with 1 *N* NH_4OH, and extracted twice with 5 ml of butyl chloride. The organic fractions were combined, 1 ml of 0.1 *N* HCl was added, and the solution was shaken for 20 min. Then the organic layer was discarded, and the aqueous phase was adjusted to pH 9 with 0.4 *N* NH_4OH and extracted twice with 5 ml of butyl chloride. The organic layers were combined and dried, and the solvent was evaporated under nitrogen at 40°.

Recovery of etorphine added to control rabbit urine was studied using $^3H_2$-etorphine, 36,000 cpm.

Formation of Derivatives—Trimethylsilyl derivatives were formed by reacting the residue with 100 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (9) at 60° for 0.5 hr. Different amounts of a mixture of etorphine and tritiated etorphine were taken and derivatized similarly. The reaction mixtures were taken to dryness under a nitrogen stream and then dissolved in 20 μl of benzene. An aliquot of this solution was injected in the gas chromatograph–mass spectrometer for the mass fragmentographic assay.

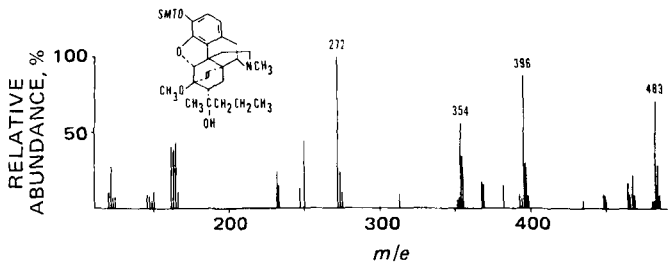

Figure 1—*Mass spectrum of etorphine trimethylsilyl (SMT) ether.*

Instrumentation—A magnetic sector, single focusing mass spectrometer[4] interfaced with a gas chromatograph and equipped with a multiple-ion detector/peak matcher accessory was used (7, 8).

GLC was performed on a 1.8-m × 2-mm i.d. column silanized before packing with 5% dimethylchlorosilane in toluene[3] and packed with 1% OV-1 on 100–120-mesh Gas Chrom Q[5]. The column was conditioned for 24 hr at 280° with a flow rate of 20 ml of helium/min. The column temperature was 230°, the flash heater was at 250°, the separator was at 250°, the ion source was at 270°, and the trap current was at 60 μamp.

In the scan mode, the accelerating voltage was 3.5 kv and the ionization potential was 70 ev. The magnetic field was kept constant by focusing the background peak at *m/e* 429, and the voltages were 3082 and 3108 v for measuring the ion intensities at *m/e* 487 and 483, respectively. The retention time was 5.5 min.

RESULTS

Recovery of etorphine added to control urine was studied using $^3H_2$-etorphine. In a typical experiment, $^3H_2$-etorphine (36,000 cpm) was added to control urine and extracted as described; 23,240 cpm was recovered. Based on 10 determinations, a mean recovery of 68 ± 6% (*SD*) was obtained for $^3H_2$-etorphine added to urine. On treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide under the experimental conditions,

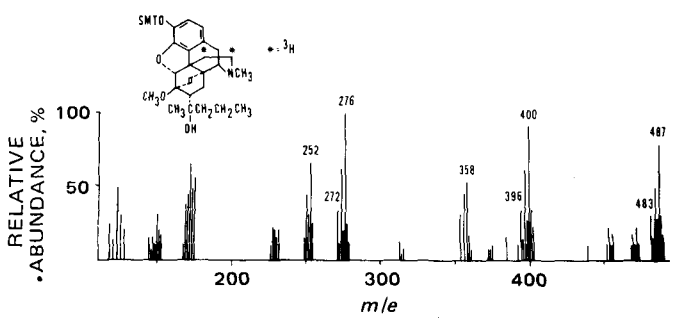

Figure 2—*Mass spectrum of tritiated etorphine trimethylsilyl (SMT) ether. The molecular ion at* m/e *487, with two tritium atoms, shows the highest relative intensity and was used for the mass fragmentographic assay.*

[1] Research Triangle Institute, Research Triangle Park, N.C.
[2] Amersham/Searle, Amersham, England.
[3] Pierce Chemical Co., Rockford, Ill.
[4] LKB model 9000.
[5] Applied Science Laboratories, State College, Pa.

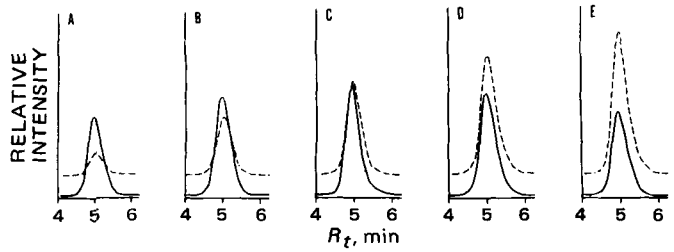

Figure 3—*Mass fragmentograms obtained by injecting approximately 5% (1 μl) of a solution containing 0, 8, 16, 24, and 40 ng of etorphine (A–D, respectively). Each solution contained 25 ng of $^3H_2$-etorphine. Key: - - -, etorphine trimethylsilyl ether, m/e 483; and —, $^3H_2$-etorphine trimethylsilyl ether, m/e 487.*

etorphine yielded a single trimethylsilyl derivative. The mass spectrum (Fig. 1) showed a molecular ion at *m/e* 483, and the fragmentation pattern is consistent with the structure.

The spectrum of tritiated etorphine derivative (Fig. 2) showed a molecular ion at *m/e* 487 and ions at *m/e* 483 and 485. The ion at *m/e* 487, corresponding to two tritium atoms, showed the maximum relative intensity and was used for quantitation. Known mixtures of etorphine, 0–40 ng per "fixed" amount (25 ng) of internal standard, were each added to 2 ml of control urine. Samples were extracted, derivatized, and analyzed on the gas chromatogram–mass spectrometer focused on ions 483 and 487. Figure 3 illustrates the mass fragmentograms, showing linear increase in the ion intensity ratio 483/487 with an increasing amount of etorphine.

The calibration curve was calculated from the data presented in Fig. 3 and was used for quantitation of etorphine present in the urine, with correction for the presence of small amounts of cold etorphine in the internal standard (10). The assay was reproducible to ±7% at the 5-ng level, and the reproducibility was much better at higher levels. The practical sensitivity of the assay in its current form is about 5 ng/ml of urine, which compares with a sensitivity of 100 ng/ml for GLC (6). Obviously, with ordinary GLC, the sensitivity would have been inadequate in the present experiments, since the maximum concentrations found were 50 ng/ml.

A number of experiments were performed on rabbits to determine urinary excretion after the subcutaneous administration of varying doses of etorphine. Figures 4 and 5 show the urinary etorphine excretion after the subcutaneous administration of 25 μg of etorphine hydrochloride in water to a 6-kg rabbit. Urine was collected by catherization periodically at 2-hr intervals for 8 hr after the dose was given. The last sample was collected 24 hr after the injection.

DISCUSSION

The specific activity of commercially available etorphine makes it possible to establish a mass fragmentographic assay for etorphine without

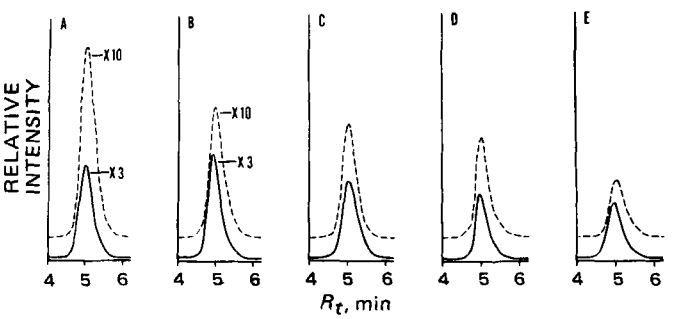

Figure 4—*Mass fragmentograms obtained from 2-ml aliquots of each urine sample collected over 24 hr and processed as described. Mass fragmentogram A represents 12% of total urine volume; B, C, and D each represent 33% of total urine volume; and E represents 25% of total urine volume. Key: - - -, rabbit urine etorphine trimethylsilyl ether, m/e 483; and —, $^3H_2$-etorphine trimethylsilyl ether, m/e 487.*

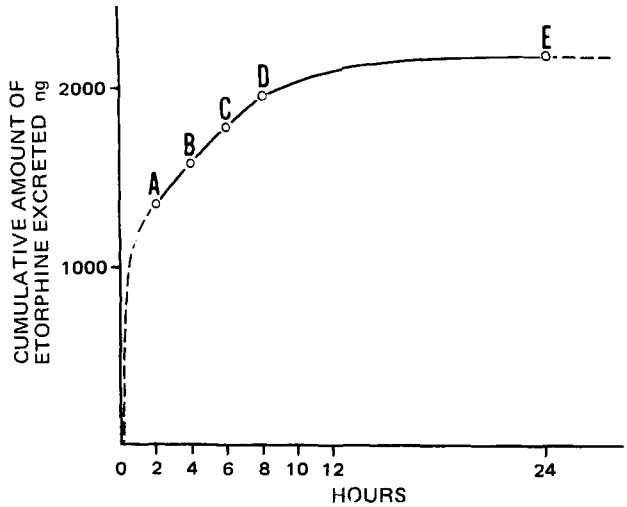

Figure 5—*Cumulative urinary excretion of etorphine over 24 hr.*

synthesis of a deuterated standard. The method could possibly be twice as sensitive if a fully deuterated standard can be obtained. This method does have the advantage that it can be immediately established by any laboratory having a gas chromatograph–mass spectrometer.

The fact that a radioisotope is used made it possible, during method development, to monitor each step of the analytical procedure. The use of small amounts of tritiated compounds in the mass spectrometer poses no problem if reasonable safety procedures are followed with proper exhaustion from the vacuum lines and proper precautions when oil is changed in the vacuum pumps. The advantage of an isotopically labeled internal standard otherwise chemically identical to the substance to be assayed is that it can be expected to pass through the different physicochemical steps of the analysis in a way similar to the compound itself to compensate for losses.

The sensitivity of the selective ion monitoring assay can probably be improved by prepurification of the biological sample or by purification and concentration by liquid chromatography of the trimethylsilyl derivative just prior to GLC–mass spectrometry.

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