High-Performance Liquid Chromatographic Determination of Diethylpropion Hydrochloride in Tablets: Isolation and Identification of Two Decomposition Products

MILDA J. WALTERS and STEPHEN M. WALTERS ˣ

Abstract □ A rapid assay was developed for diethylpropion hydrochloride tablets using high-performance liquid chromatography (HPLC) with UV detection. This technique provided separation of the drug from other UV-absorbing components present as the result of decomposition. A major decomposition product detected by HPLC in extracts of tablets and of the cotton filler from a tablet bottle was collected from the column effluents. This product was subsequently identified as 1-phenyl-1,2-propanedione, a highly volatile compound. A second decomposition product, isolated from decomposed drug by distillation from alkaline solution, was identified as diethylamine, apparently present as the hydrochloride salt. GLC, UV, IR, NMR, and mass spectrometry were used to confirm the identity of the decomposition products. HPLC assay results compared favorably with results of the NF assay; the latter procedure separates the drug from 1-phenyl-1,2-propanedione *via* liquid–liquid extraction.

Keyphrases □ Diethylpropion hydrochloride—high-performance liquid chromatographic analysis in tablets, identification of two decomposition products □ High-performance liquid chromatography—analysis, diethylpropion hydrochloride in tablets, identification of two decomposition products □ Anorectic agents—diethylpropion hydrochloride, high-performance liquid chromatographic analysis in tablets, identification of two decomposition products □ Decomposition products—of diethylpropion hydrochloride, identification, high-performance liquid chromatographic analysis in tablets

Diethylpropion has been reported to be an effective anorectic agent (1–3). A GLC method for the analysis of diethylpropion and its basic metabolites in urine was described previously (4). The metabolites investigated in that study (4) and in a later study (5) consisted of amino ketones and amino alcohols formed by *N*-dealkylation and keto reduction. Another study (6) detected 23 metabolic products of diethylpropion in human urine, of which 21 were identified. Two major products remained unidentified.

The investigation of possible decomposition of diethylpropion hydrochloride (I) in tablets was prompted by a disparity in analytical results obtained during the assay of several samples. Results obtained by the NF UV spectrophotometric procedure (7) were significantly lower than the monograph requirements. However, assays of extracts of these samples by perchloric acid titration gave results near the labeled amounts. Therefore, it was assumed that molecular dissociation had occurred, leaving the diethylamine group (reactant with perchloric acid) separated at some point from the remaining UV-absorbing portion. This assumption was further evidenced by the discovery of a yellow, highly volatile, UV-absorbing material in the cotton filler of a freshly opened bottle of tablets.

A high-pressure liquid chromatography (HPLC)–UV detection method was developed to provide a relatively rapid assay of I in tablets. It provided an additional advantage of isolating I from other UV-absorbing components present as the result of decomposition. GLC, UV, IR, NMR, and mass spectrometry were subsequently used to identify two decomposition products, 1-phenyl-1,2-propanedione (II) and diethylamine as the hydrochloride (III).

A report of the formation of II as a product of I has not been found, and the mechanism involved remains to be elucidated. The formation of diethylamine, in addition to ethylamine, acetaldehyde, and propiophenone, was reported as a result of photo- (254 nm) and radium-irradiation of aqueous and methanolic solutions of I (8).

EXPERIMENTAL

Instrumentation—*HPLC*—The liquid chromatograph[1] was equipped with a variable wavelength spectrophotometric detector[2] and a 2.6-mm i.d. × 50-cm stainless steel column containing a chemically bonded octadecylsilane on silica (13 ± 5 μm particle size) reversed-phase packing[3]. The detector wavelength was set at 255 nm, and the sensitivity was adjusted to produce a peak height of approximately 100 mm for 1.25 μg of I on a 50-mv recorder[4].

Two mobile solvent systems were used: A, a mixture containing 70% acetonitrile, 10% methanol, and 20% aqueous 0.1% ammonium carbonate; and B, a mixture containing 35% acetonitrile, 5% methanol, and 60% aqueous 0.1% ammonium carbonate. The flow rate was 1.0 ml/min, and elutions were made at ambient temperature. The retention volumes (V_R's) of I and II were 5.2 and 2.7 ml, respectively, using System B. The V_R of the internal standard, procaine hydrochloride, was 10.5 ml using System A for the quantitation of I.

GLC—A gas chromatograph[5] equipped with a flame-ionization detector was used. Compound II was chromatographed on a 2-mm × 1.83-m (6-ft) glass column packed with 16% DC 200 on Chromosorb WHP (80–100 mesh), with injector, column, and detector temperatures at 200, 100, and 250°, respectively. The helium carrier gas flow was 90 ml/min, and the detector sensitivity was 8×10^{-10} amp/mv. The retention time of II was 7.4 min.

Compound III was chromatographed on a 2-mm × 1.83-m (6-ft) glass column packed with 20% Carbowax 20M on Chromosorb WHP (80–100 mesh), with injector, column, and detector temperatures at 150, 60, and 250°, respectively. The helium carrier gas flow rate was 24 ml/min, and the detector sensitivity was 8×10^{-9} amp/mv. The retention time of III was 1 min.

IR—The IR spectrum of neat II was obtained[6] between sodium chloride plates, scanning from 2.5 to 16 μm. The spectrum of III was obtained[7] in the vapor phase in a 10-cm gas cell, scanning from 2 to 14 μm.

UV—The UV spectra of I and II were obtained[8] using 1-cm cells, scanning from 350 to 200 nm.

NMR—The NMR spectrum of II was obtained[9] in deuterated chloroform, scanning from 0 to 9 ppm.

Mass Spectrometry—The mass spectrum of II was obtained on a magnetic deflection mass spectrometer[10]. The sample was introduced *via* the liquid–solid direct insertion probe and ionized at a potential of 70 ev.

HPLC Determination of I in Tablets—A portion of finely ground

[1] Perkin-Elmer model 1220.
[2] Perkin-Elmer model LC-55.
[3] ODS SIL-X-1, Perkin-Elmer Corp., Norwalk, CT 06856.
[4] Leeds & Northrup Speedomax X/L 680.
[5] Perkin-Elmer model 990.
[6] Beckman model IR 20.
[7] Perkin-Elmer model 21.
[8] Cary model 118.
[9] Perkin-Elmer model R12B.
[10] Hitachi Perkin-Elmer model RMS-4.

Table I—Assays of Drug Substance and Commercial 25-mg Tablets[1]: Comparison of Titrimetric, NF, and HPLC Methods

Sample	Diethylpropion Hydrochloride, % of Labeled Amount Found		
	Titrimetric	NF	HPLC
A (tablets)	101.0	88.6	—
B (tablets)	96.8	86.2	—
C (tablets)	—	82.4	81.7[a]
D (tablets)	—	80.8	83.6[a]
E (tablets)	96.8	97.2	96.3 ± 0.53[b]
F (drug substance)	98.9	98.9	99.9

[a] Average of three determinations. [b] Average ± *SD* of six determinations.

tablet composite equivalent to 25 mg of I was weighed into a 50-ml volumetric flask, and 25 ml of methanol was added. The flask was placed in an ultrasonic bath[11] for 10 min, and then the solution was diluted to volume with methanol. The solution was filtered through fast filter paper, the first 10 ml of filtrate was discarded, and a 2-ml aliquot was mixed with 2.0 ml of procaine hydrochloride internal standard solution (2 mg/ml in methanol). A standard solution of I was prepared by mixing 2.0 ml of a stock solution (0.5 mg/ml in methanol) with 2.0 ml of internal standard solution.

Five-microliter injections of these solutions were made into the liquid chromatograph using mobile Solvent System A. When the peak height ratios (*PHR*) of I to the internal standard were reproducible within ±1% of the average for at least three consecutive injections of the standard

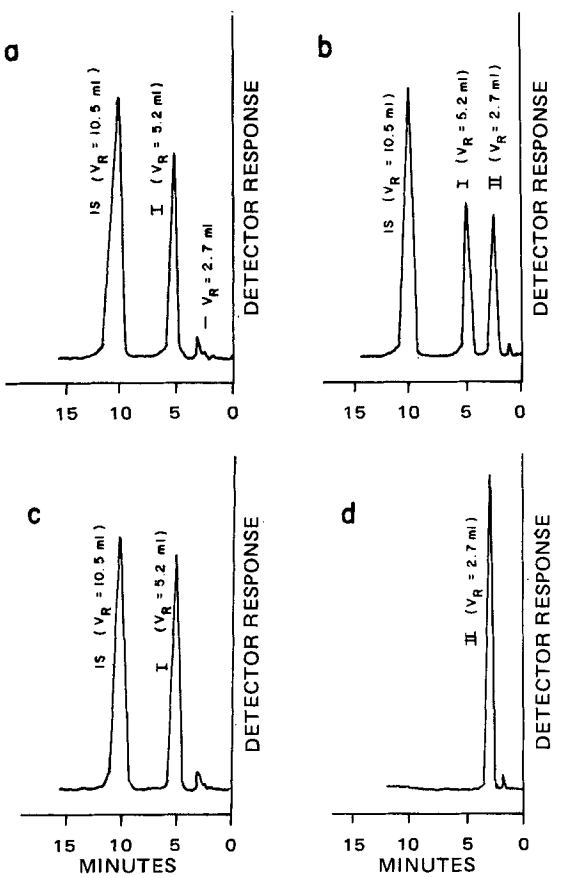

Figure 1—*HPLC separation of diethylpropion hydrochloride (I), decomposition product 1-phenyl-1,2-propanedione (II), and internal standard procaine hydrochloride (IS). Chromatograms are: a, an extract of a freshly prepared composite of undecomposed tablets; b, an extract of an aged, decomposed tablet composite; c, a standard solution of I; and d, an extract of yellow material from a cotton filler removed from a tablet bottle.*

[11] Bransonic 32, Branson Instruments Co., Shelton, CT 06484.

Table II—Effects of Elevated Temperature and Humidity on Diethylpropion Hydrochloride Assay Results

Sample	Conditions			Percent of Labeled Amount Found[a]	Method
	Temperature	Humidity	Hours		
D (tablets)	50°	Dry	48	98.0	HPLC
	50°	Dry	144	96.0	HPLC
	50°	Dry	744	88.8	HPLC
	50°	Humid	72	74.4	NF
				84.4	Titrimetric
	50°	Humid	960	52.4	NF
				82.4	Titrimetric
E (drug substance)	50°	Humid	36	94.7	HPLC
	50°	Humid	528	90.0	NF
				105.0	Titrimetric

[a] All results are corrected for weight increases due to absorption of water.

solution, alternate sample and standard injections were made and the concentration of I in the tablets was calculated as:

$$\frac{PHR_{spl}}{PHR_{std}} \times \text{standard concentration, mg/ml} \times \text{dilution of sample (50 ml)} \times \frac{\text{average tablet weight, g}}{\text{weight of sample, g}} = \frac{\text{mg of I}}{\text{tablet}} \quad \text{(Eq. 1)}$$

Titrimetric Determination of I in Tablets—A portion of tablet composite equivalent to 200 mg of I was weighed and transferred into a separator containing 25 ml of water. The solution was made alkaline with 0.1 *N* NaOH and extracted with three 50-ml portions of chloroform. The extracts were filtered through a funnel containing a cotton plug covered with 5 g of anhydrous sodium sulfate. To the filtrate was added 25 ml of acetic acid, and the solution was titrated with 0.1 *N* perchloric acid in acetic acid, using 2 drops of gentian violet solution (10 mg/ml in acetic acid) as indicator.

Isolation of II from Tablets and Cotton Fillers—The yellow substance found in the cotton fillers in the tablet bottles and a portion of an aged tablet composite were both extracted with methanol, concentrated, and injected into the liquid chromatograph. Retention volumes were measured and compared using both Solvent Systems A and B. Fractions of the eluant corresponding to the equal retention volumes of the major peak in the cotton extract chromatogram and of the extraneous peak in the tablet extract chromatogram were collected from System B. UV spectrophotometric measurements were made directly on the collected eluants. The eluants were then extracted into pentane and concentrated by evaporation for GLC, IR, and mass spectrometric analyses. The volatility of the collected material proved to be a problem in isolating sufficient amounts for IR and mass spectrometric analyses. A satisfactory mass spectrum of the cotton extract only was obtained.

Accelerated Production of II—In an attempt to collect larger amounts of this unknown component for further analysis, accelerated degradation of I (drug substance) was performed. The most efficient method found was to place 1–2 g of the drug substance in an erlenmeyer flask with 1–2 ml of water, connecting the flask to a distilling adapter with the tip immersed in distilled water contained in a second erlenmeyer flask, and placing the first flask on a steam bath while maintaining the second flask near room temperature. After collecting the volatile product in this manner over 3–4 days, the unknown component was isolated from the water solution by extraction into pentane; very careful evaporation of the solvent yielded a yellow liquid residue.

Stability Study on Tablets and Drug Substance—A limited study of the effects of elevated temperature and humidity on the stability of I in both tablet and drug substance form was performed. Portions of previously assayed samples were weighed into small beakers. Some portions were subjected to an elevated temperature condition by placing them in an oven at 50°. The remaining portions were subjected to humid, elevated temperature conditions by placing them in a large beaker containing a smaller beaker of water, covering the large beaker with aluminum foil, and placing it in an oven at 50°.

The samples were assayed at various times by the NF or HPLC method, and some were assayed by the titrimetric method as well. All results were corrected for weight increases due to water absorption.

Isolation of III from Decomposed Drug Substance—To a portion

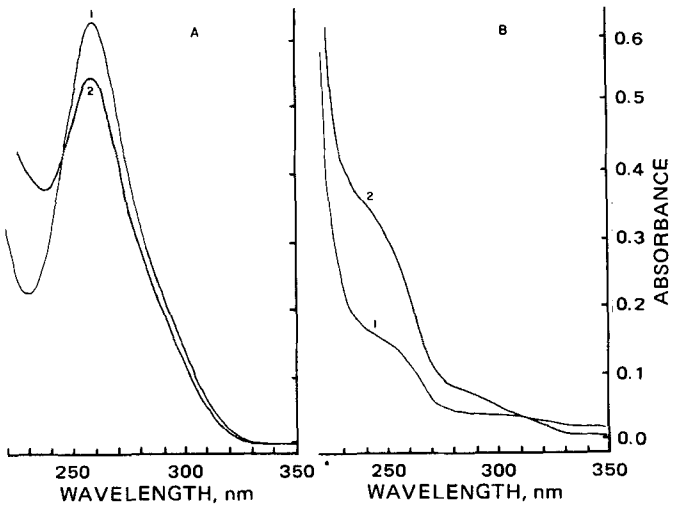

Figure 2—*UV spectra of (1) 1-phenyl-1,2-propanedione reference material and (2) isolated decomposition product II. A shows spectra obtained in water, and B shows spectra obtained after addition of sodium hydroxide.*

(~0.5 g) of decomposed drug substance remaining from the stability study was added 10 ml of 1 *N* NaOH. This solution was distilled from a microdistillation apparatus[12] by placing the distilling flask on a steam bath until several drops (~0.5 ml) of distillate were collected.

A portion of the aqueous distillate was extracted with chloroform for GLC analysis. Another portion was placed in a gas cell and warmed briefly to induce vaporization just prior to obtaining the IR spectrum.

RESULTS AND DISCUSSION

The assumption that partial decomposition of I in tablets had occurred *via* molecular dissociation of the amine group and the UV chromophore, with a rearrangement of the latter to a volatile compound, is evidenced by the comparative results of the titrimetric and NF or HPLC assays (Table I). This assumption was further evidenced by the finding of a foreign, UV-active, yellow material in a cotton filler, which was examined immediately upon removal from a bottle of tablets.

The volatile decomposition product exhibited UV activity with maximum absorbance near that of I. Thus, the agreement of results between the NF and the specific HPLC assays suggests that the volatile decomposition product (II) had escaped entirely from the tablet samples prior to performing the NF assays or that isolation of I from any remaining residues of II occurred in the liquid–liquid extraction steps of the NF procedure. A subsequent investigation found that the latter would occur.

The results of the limited stability study (Table II) indicate a significantly faster rate of decomposition with increased humidity, suggesting that hydrolysis may be involved in the process.

The relative values obtained by the NF and titrimetric assays of the decomposed samples again reflect the loss of UV-absorbing material in the decomposition process.

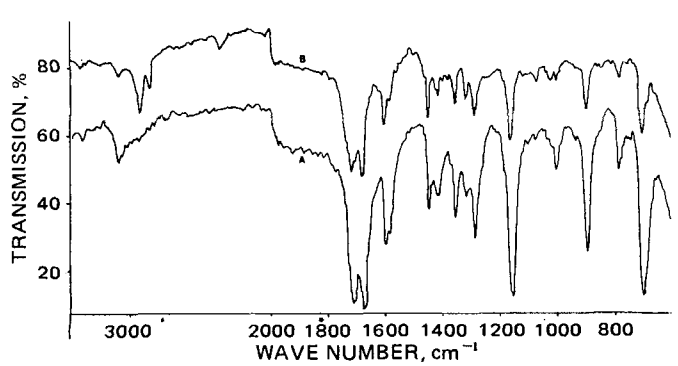

Figure 3—*IR spectra of (A) 1-phenyl-1,2-propanedione reference material and (B) isolated decomposition product II.*

[12] Ace Glass Inc., Vineland, N.J.

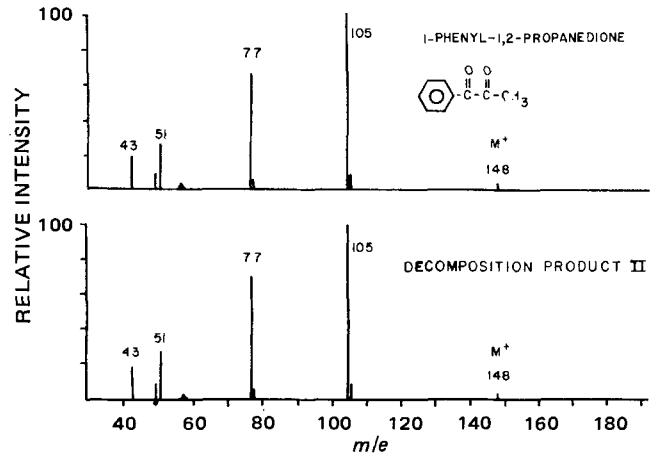

Figure 4—*Mass spectra of 1-phenyl-1,2-propanedione reference material and isolated decomposition product II.*

Typical liquid chromatograms obtained using Solvent System A are shown in Fig. 1. The aged tablet composite chromatogram contains an extraneous peak with the same V_R as the peak seen in the cotton filler chromatogram. This peak is absent in the standard and fresh composite chromatograms.

The liquid chromatograms obtained for the aged tablet composite and the cotton filler using Solvent System B also show an extraneous peak in the former with the same V_R as the peak seen in the latter. The use of this solvent system provided a larger V_R than did System A for the extraneous peak, with greater resolution from minor unknown peaks (probably decomposition by-products), thus permitting greater selectivity in the fraction collection process.

Subsequent HPLC analysis of the laboratory-produced decomposition product resulted in chromatograms similar to those of the cotton filler extract for both solvent systems.

The gas chromatograms and UV spectra showed identical characteristics for the laboratory-produced material and the collected HPLC fractions of the cotton filler and aged tablet composite extracts. The UV spectra consisted of a single maximum at about 255 nm when scanned in methanol or water, but it changed to a shoulder after addition of sodium hydroxide (Fig. 2).

The mass spectra of the laboratory-produced material and the collected fraction of the cotton extract showed identical fragmentation patterns, with a molecular ion peak at *m/e* 148 and major peaks at *m/e* 105, 77, 51, and 43.

The IR spectrum of the laboratory-produced material showed a double carbonyl band in the 1700-cm^{-1} region. A lack of bands in the 3500–

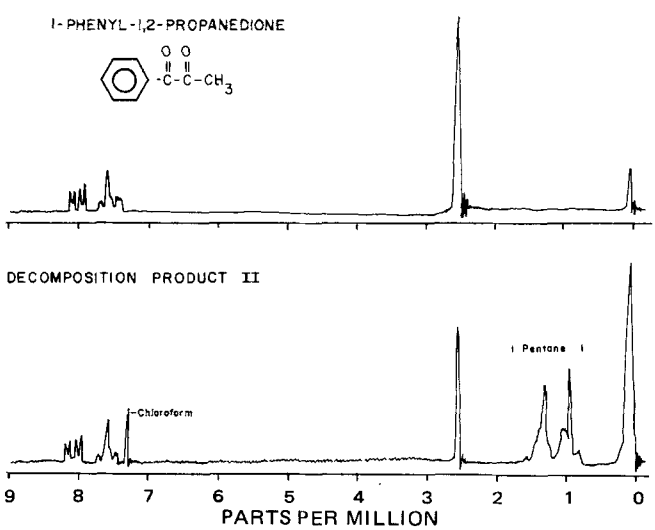

Figure 5—*NMR spectra of 1-phenyl-1,2-propanedione reference material and isolated decomposition product II.*

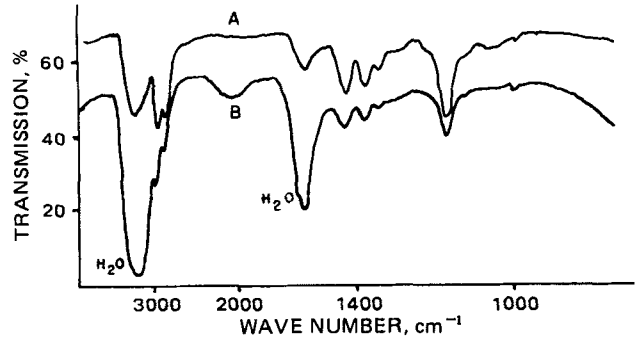

Figure 6—*IR spectra of (A) diethylamine reagent and (B) isolated decomposition product III.*

3000-cm^{-1} region indicated the absence of hydroxyl and primary or secondary amine groups.

The mass and IR spectra provided sufficient evidence for the elucidation of the structure of II. This compound was available commercially[13] and was purchased to serve as a reference material. The UV spectra of this reference material were nearly identical to those of the laboratory-produced decomposition product (Fig. 2). However, some background absorbance was apparent in the latter.

The IR and mass spectra of these two materials appeared identical (Figs. 3 and 4). The NMR spectra (Fig. 5) were also identical, with the exception of pentane peaks in the spectrum of the laboratory-produced material due to a residue remaining from the extraction step. A peak due to a chloroform impurity in the deuterated solvent was also visible in this spectrum because of the higher instrument gain used.

Gas and liquid chromatograms subsequently obtained for the reference material showed peaks identical to those of the previously unknown component. The chromatographic and spectral data combined with the identical physical characteristics of the cotton filler extract, the laboratory-produced material, and the reference material provided convincing evidence that II was formed as a major decomposition product of I.

Prior to the distillation of III from decomposed drug substance, the latter material was tested and found positive for secondary amines by the NF monograph test. The identity of III as a second decomposition product was then established by comparison of the gas chromatogram and the IR spectrum of the distillate with those of the diethylamine reagent[14]. Water was added to the latter prior to vaporization in the IR cell to simulate better its effect on the isolate spectrum (Fig. 6).

The distillate of an alkaline solution of undecomposed drug substance was found to contain no diethylamine, thus eliminating the suspicion that the latter may have been formed during the isolation procedure.

The route of decomposition from I to II and III has not been satisfactorily elucidated at this time and is apparently quite complex. However, it has been demonstrated that this type of decomposition has occurred in commercial tablet products and that it is accelerated by the presence of moisture.

The NF monograph assay procedure is specific for I in the presence of II, since it was found that II is retained in the sodium hydroxide solution while I is extracted into chloroform. However, the content uniformity procedure involves the direct UV determination of filtered hydrochloric acid solutions of the tablets. Since II was found to have considerable absorptivity and a maximum at 257 nm in this solvent, its presence in tablets could be expected to increase significantly the absorbance readings of their solutions, leading to inaccurately high results.

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Identification of Diacetylmorphine Metabolites in Humans

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Abstract □ With the techniques of column chromatography, TLC, and GLC, morphine, 6-acetylmorphine, normorphine, morphine 3-glucuronide, 6-acetylmorphine 3-glucuronide, and normorphine glucuronide were identified as metabolites of diacetylmorphine (heroin) in the urine of humans administered 10 mg iv/70 kg body weight.

Keyphrases □ Diacetylmorphine—metabolites identified using TLC, GLC, and column chromatography, human urine □ Metabolites—diacetylmorphine, identified using TLC, GLC, and column chromatography, human urine □ TLC—identification, diacetylmorphine metabolites, human urine □ GLC—identification, diacetylmorphine metabolites, human urine □ Column chromatography—identification, diacetylmorphine metabolites, human urine □ Narcotics—diacetylmorphine, metabolites identified using TLC, GLC, and column chromatography, human urine

Studies in laboratory animals *in vitro* and *in vivo* indicated that diacetylmorphine (heroin) (I) was rapidly metabolized first to 6-acetylmorphine and then to morphine (1–5). In humans, diacetylmorphine was excreted in the urine mainly as morphine (6, 7). Approximately 50–60% of I administered to heroin addicts was excreted in the urine as conjugated morphine and 7% as free morphine (8, 9). In addition to free and conjugated morphine, small