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Re-evaluation of the metabolism and excretion of diethylpropion in non-sustained and sustained release formulations

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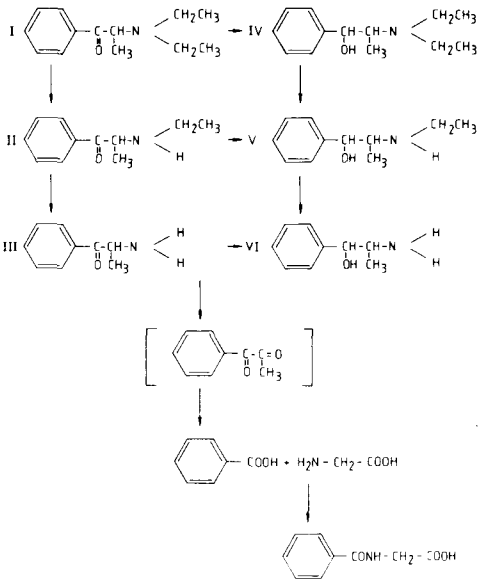
A direct gas chromatographic method for unstable amino ketones, using a neutral column and moderately alkaline conditions during extraction has been developed. Its application has given a new perspective to the relative importance of the metabolic routes in the complex metabolism of diethylpropion which after oral administration in man is rapid and extensive (only 3–4% of the drug remains unchanged). Mono-*N*-de-ethylation is the main pathway (about 35% of the dose). *N*-De-ethylation is more important than carbonyl-reduction, occurring mainly with the unchanged drug (about 20% of the dose). Norephedrine, thought previously to be one of the main metabolites, has been shown to be present only in negligible amounts. About 30% of the dose, which cannot be accounted for as the sum of the amines recovered in urine, is probably metabolized by deamination, followed by oxidation and conjugation to give hippuric acid.

The metabolism of diethylpropion and the excretion of the drug and its metabolites in man has been investigated by several researchers (Schreiber et al 1968; Banci et al 1971; Hossie 1970; Testa & Beckett 1972, 1973, 1974; Wright et al 1975). However, none of these studies completely resolved the metabolism and excretion of diethylpropion. The complex metabolic picture (see Scheme), and the difficulties of separating the compounds, some of which are unstable, has been the reason for different interpretations and wide discrepancies.

Schreiber et al (1968) examined the metabolism of [1-^{14}C]diethylpropion in man using TLC. They found that the radiolabel was excreted exclusively via the renal pathway, and that extensive metabolic alterations of the drug, including aromatic hydroxylation, *N*-de-ethylation and reduction, had occurred. Twenty-one metabolic products were identified in urine that had been adjusted to pH 12 and extracted with diethyl ether. Hippuric acid (about 27% of the radioactivity excreted in the urine) was found as the metabolite present in the greatest quantity. Only small amounts of amino ketones were found, probably due to the extraction procedure carried out.

A GC method for the investigation of the metabolism and excretion of diethylpropion was developed by Banci et al (1971) who found carbonyl-reduction of the parent compound (metabolite IV) to be the main metabolic pathway in man. However, they mentioned that 2-ethylaminopropiophenone (metabolite II) was excreted in an amount similar to that of metabolite IV, and concluded that *N*-de-ethylation and reduction were the main metabolic pathways of diethylpropion in man. Taking into consideration the analytical conditions used (KOH-coated GC columns and extraction in the presence of 5 M NaOH), the results obtained are understandable.



Scheme: Metabolism of diethylpropion.

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The work of Hossie (1970), and that of Testa & Beckett (1972) resulted in a GC method for the analysis of diethylpropion and its basic metabolites in urine. The same procedure was originally used to study the metabolism and excretion of 4'-chloro-2-ethylaminopropiophenone and its metabolites (Beckett & Hossie 1969a, b). The method described by Testa & Beckett (1972) determined the amino alcohol metabolites in the presence of the amino ketone metabolites, as "the latter could not be determined directly because of decomposition during extraction and on the column". The amino ketone metabolites were determined "after quantitative reduction to corresponding amino alcohols". The authors stated that there was no interference of amino ketones I, II and III during analysis of amino alcohols IV, V and VI (Fig. 1).

Wright et al (1975), using four complementary analytical procedures, examined diethylpropion and its metabolites in blood plasma from oral or subcutaneous doses. However, they were not able to measure norephedrine (metabolite VI) in plasma. They showed slightly delayed metabolism of diethylpropion (measured by appearance of metabolite II), and much higher concentrations of unchanged drug after subcutaneous than after oral administration.

As the method of Testa & Beckett (1972) for analysis of diethylpropion and its metabolites seemed to be one of the most complete procedures, an unsuccessful attempt was made to adapt it to our needs. By GC and GC–MS it was shown that amino ketones decomposed on KOH-treated columns in a way different from that in the presence of an alkali, and only to a limited extent (Beckett & Stanojčić 1983). Consequently, large co-extraction from alkaline medium and incomplete decomposition on the column of amino ketones occurred giving incorrect results for diethylpropion and its metabolites.

A new GC procedure for the analysis of diethylpropion and its basic metabolites was therefore developed, and used to re-evaluate the drug's metabolism and urinary excretion.

MATERIALS AND METHODS

Compounds

The compounds were kindly supplied by Temmler-Werke, Marburg/Lahn (*N*-diethylaminopropiophenone–diethylpropion HCl, I; *N*-ethylaminopropiophenone HCl, II; amino-propiophenone HCl, III and *N*-ethylnorephedrine HCl, V); Wm. S. Merrell Co., Cincinnati, Ohio, USA (*N*-diethylnorephedrine, IV); Ralph N. Emanuel Ltd, Wembley, UK (norephedrine HCl, VI) and Laboratories Diamant S.A., Paris (furfurylamphetamine acetate).

Gas chromatography (GC)

All the compounds were chromatographed using a PYE-104 instrument which incorporated a flame ionization detector. The systems and conditions were:

(1) A 1 m glass column (i.d. 4 mm), containing chromosorb G AW DMCS-treated (100–120 mesh) coated with 2% Carbowax 20M and 10% Apiezon L; carrier gas–nitrogen, pressure 100 kPa, flow-rate 1·25 $cm^3 s^{-1}$; air and hydrogen pressures 135 kPa; column, injector and detector temperatures 200 °C.

(2) A 1 m glass column, containing the same material as column 1 but also coated with 10% KOH, and working under the same conditions.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS spectra were recorded on a VG 12F mass spectrometer with VGDS 2135 data system (ionization potential 70 eV) linked to a PYE-104 gas chromatograph (system 2).

Direct inlet mass spectra (electron impact mode) of the compounds examined were obtained on the same instrument at an ionization potential of 70 eV.

Extraction procedure

(a) *Determination of compounds I, II, IV and V (GC system 1).* To 4 mL of urine, 1 mL of internal standard solution (I.S.) (furfurylamphetamine acetate, 40 μg mL^{-1}) was added. The mixture was made alkaline (pH 10·5) with 0·5 mL of 30% v/v NH_4OH (s.g. 0·88), and extracted with freshly distilled diethyl ether (3 × 4 mL) by shaking gently for 15 min and centrifuging for 5 min after each extraction. The ether extracts were transferred to a 15 mL Quickfit test tube (tapered base) and concentrated to ca 50 μL (in a water bath, 40 °C). The tube was placed in ice and 3–5 μL were analysed by GC.

Calibration curves of compounds I, II, IV and V were obtained by analysing blank urine samples containing known amounts of the compounds (0·68–6·80 μg base mL^{-1} of I and IV, and 0·80–8·00 μg base mL^{-1} of II and V). The concentrations in the urine were obtained by using their peak height ratios to the I.S.

(b) *Determination of compounds III and VI (GC system 2).* To 4 mL of urine, 1 mL of I.S. solution (furfurylamphetamine acetate, 40 μg mL^{-1}) was added and the tube was placed in ice. Sodium borohydride (ca 30 mg) was added, the tube slowly

inverted once and returned to the ice for 20 min. The reduction process was repeated twice at 20 min intervals. The urine was saturated with 2 g of NaCl and then 0·5 mL of 5 M NaOH was added, and the extraction was carried out with 4 × 4 mL of freshly distilled diethyl ether. Pooled ether extracts were concentrated to ca 50 μL as described under (a) and analysis was performed using GC.

The calibration curve of compound VI was obtained by analysing blank urine samples containing known amounts of compound III (0·64–6·46 μg base mL^{-1}) in the same way as described for urine samples under (b). The concentrations of VI were obtained by using the peak height ratios to the I.S.

Urinary excretion trials

Three healthy subjects (one female and two males) took the drug orally on an empty stomach. The subjects were screened both medically and biochemically before the study, and each of them signed informed consent. The study was approved by the local ethical committee.

The dose was either 25 mg of diethylpropion hydrochloride in aqueous solution, or 75 mg as the sustained release pellet formulation (SRF, lot 22-A-78). The subjects were non-smokers, since it was confirmed that nicotine interfered with the analysis of the metabolite(s) III and/or VI. All doses were taken in 50–100 mL of water. Control urine samples were collected from the subjects just before each dose. When 25 mg of diethylpropion hydrochloride was given, urine was collected every ½ h for 4 h, hourly for the next 8 h and then every 2–3 h over the remainder of the 30 h period. When 75 mg of the drug was given, urine was collected hourly the first day, and every 2–3 h over the remainder of the 48 h.

Acidic urine (pH 4·9 ± 0·2) was induced and maintained by ammonium chloride sustained release pellets given in suitable regimens (6–8 g over 24 h). The subjects did not take food for about 12 h before they took the drug, and for about 4 h after the drug.

RESULTS AND DISCUSSION

After the ingestion of diethylpropion, urine samples were analysed directly by the method of Testa & Beckett (1972) for alcohol metabolites (IV, V and VI) content, and indirectly for ketone metabolites (I, II and III) content, by reducing the latter in solution to produce the alcohols IV, V and VI, respectively. Subtraction of the peak height ratios obtained before reduction from the peak height ratios obtained after reduction gave the ketone metabolite content.

However, using this method we obtained a large peak from the extracts for alcohol metabolites at the location for norephedrine (metabolite VI) (peak 1, Fig. 1A), and only a small peak at the same location after quantitative reduction (metabolites III + VI) (Fig. 1B). This indicated that the large peak contained a component other than a small amount of norephedrine (metabolite VI).

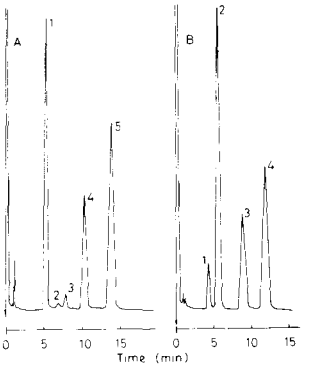

FIG. 1. The chromatograms showing analysis of the same urine sample before (A) and after reduction with sodium borohydride (B) on GC system 2 (method of Testa & Beckett 1972). (A) 1 = metabolites II, III and VI, 2 = metabolite V + decomposition product of II, 3 = diethylpropion (I), 4 = metabolite IV + decomposition product of I, 5 = internal standard. (B) 1 = metabolites III and VI (as VI), 2 = metabolites II and V (as V), 3 = diethylpropion (I) and metabolite IV (as IV), 4 = internal standard.

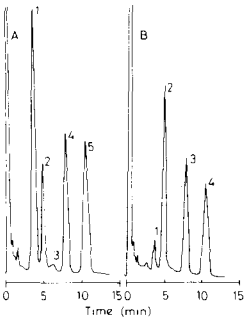

FIG. 2. Chromatogram of the compounds: I (peak 3, Rt = 7·5 min), II (peak 1, Rt = 5·2 min), IV (peak 4, Rt = 9·9 min), V (peak 2, Rt = 6·5 min) and I.S. (peak 5, Rt = 13·4 min) separated and analysed using GC system 1.

We further investigated the possibilities of directly extracting the amino ketones from the alkaline media, and their GC analysis on KOH-treated columns, and found the following:

(i) There was incomplete decomposition of amino ketones during the process of extraction from alkaline media, and therefore there was the possibility of their extraction and detection on the GC system 2 (the system used by Testa & Beckett 1972).

(ii) Decomposition of the amino ketones (not amino

alcohols) on the KOH-coated columns occurred to some extent and this process was different from decomposition in alkaline media.

(iii) Some of the amino ketones, and decomposition products of amino ketones I and II interfered with the peaks of the amino alcohols, e.g. the peak of metabolite II overlapped the peaks of metabolites III and VI; the peak of the decomposition product of metabolite II interfered with that of metabolite V, and the peak of the decomposition product of the compound I interfered with that of metabolite IV (Table 1).

Table 1. Retention times of compounds I–VI and of decomposition products of compounds I and II on the GC system 2 (nitrogen, flow rate 1·66 $cm^3 s^{-1}$).

Compounds	Rt (min)	Decomposition products
I	6·2	7·5
II	3·8	5·5
III	3·4	
IV	8·2	
V	5·1	
VI	4·0	

The amount of amino ketone II not decomposed in the procedure gave the large peak which completely swamped the smaller peaks of the amino alcohol VI and the amino ketone III which was not decomposed in the process.

To confirm this, GC–MS analysis of the peak located at the norephedrine (metabolite VI) position (peak 1, Fig. 1A) was performed, and the spectrum compared with direct inlet mass spectra of compounds II, III and VI. The GC–MS spectrum of this peak was consistent with the direct inlet mass spectrum of metabolite II: characteristic peaks in direct inlet mass spectrum of metabolite II were obtained at *m/z*: 105 (3·3%), 77 (6·8%), 72 (100%), and 51 (4·7%), and in GC–MS spectrum of the first peak, at *m/z*: 105 (29·7%), 77 (38·5%), 72 (100%), and 51 (30·7%). No fragments corresponding to metabolites III and/or VI were found.

Because of the above, a new GC method for the analysis of diethylpropion and its metabolites excreted in urine was developed. The GC system 1 was used for the analysis of the unchanged drug (I), and its metabolites II, IV and V, as it was established that amino ketones did not decompose on this column. Good separation (Fig. 2) and straight calibration curves were obtained with the concentrations used (see methods).

However, at the concentrations usually found in urine, it was not possible to detect primary amines (metabolites III and VI) on this system. Higher concentrations showed Rt of 3·8 min for metabolite III, and 4·2 min for metabolite VI. For complete information on the basic metabolites present in the urine, the analysis of primary amines III and VI was carried out on GC system 2 (after quantitative reduction to norephedrine, VI) (Fig. 3A, B).

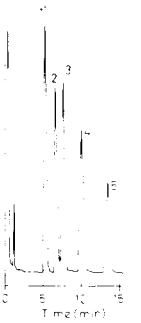

FIG. 3. Chromatograms showing analysis of the same urine sample using: (A) system 1 (1 = metabolite II, 2 = metabolite V, 3 = diethylpropion (I), 4 = metabolite IV, 5 = I.S.) and (B) system 2—after reduction with $NaBH_4$. 1 = metabolites III and VI, as VI (Rt = 4·4 min), 2 = metabolites II and V, as V (Rt = 5·7 min), 3 = diethylpropion (I) and metabolite IV, as IV (Rt = 9·1 min) and 4 = I.S. (Rt = 12·2 min).

All urine samples were analysed immediately after collection, although the compounds examined were stable in acidic urine at 4 °C for nearly two months.

The amino ketone I was stable when urine was made alkaline to pH 13 by the addition of 5 M NaOH, and immediately extracted with ether, whereas the amino ketone II was stable at pH 10·5 (urine made alkaline with 0·5 mL of 30% v/v ammonia), but decomposed about 20–25% at pH 13.

The studies on extractibility showed that the compounds examined were practically completely extracted from the urine by the extraction method chosen, i.e. at pH 10·5 using the ammonia solution (compound I: 99·4%, II: 100·3%, IV: 99·5% and V: 98·3%). The ether extracts were analysed the same day, since the extracts left at 4 °C overnight showed a decline in peak height ratios of compounds I and II of about 10%, while those of the amino alcohols IV and V remained constant.

The extraction of metabolites III and VI (as metabolite VI) was improved by saturation with NaCl, i.e. only 4 × 4 mL of ether was required, instead of 6 × 4 mL as used previously. Extractability of metabolite VI was complete (100·9%).

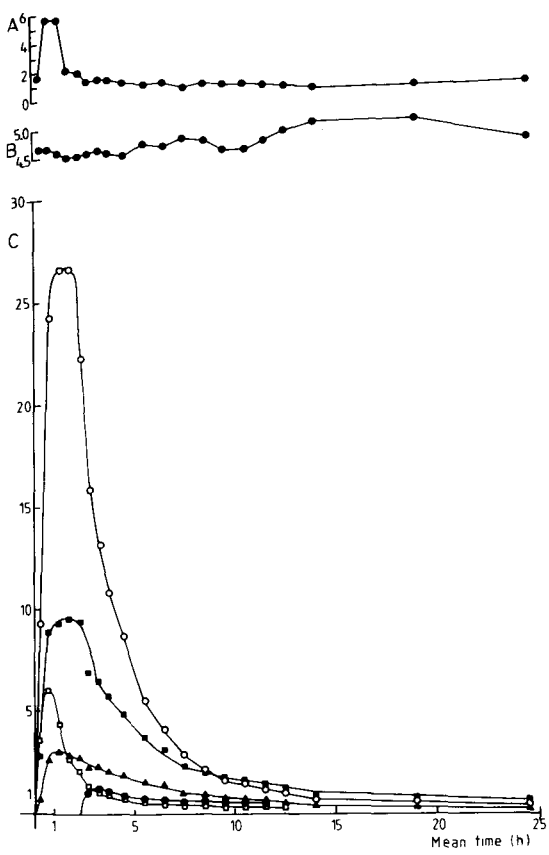

FIG. 4. Excretion in man of diethylpropion and its basic metabolites under acidic urine conditions after an oral dose of 25 mg of the hydrochloride in aqueous solution (Subject 1, b). Key: I □---□, II ○---○, IV ■---■, V ●---●, III + VI ▲---▲. A = Urine flow rate (mL min^{-1}), B = urinary pH. C = Excretion rate (μg of base min^{-1}).

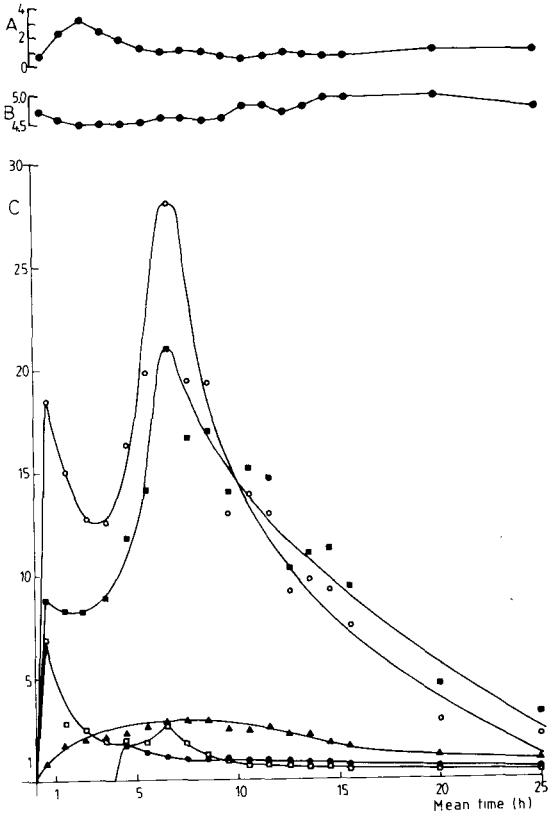

FIG. 5. Excretion in man of diethylpropion and its basic metabolites under acidic urine conditions after oral administration of sustained release pellets (75 mg of the hydrochloride). Subject 1. Key: I □---□, II ○---○, IV ■---■, V ●---●, III + VI ▲---▲. A, B, C as Fig. 4.

The borohydride reduction was carried out with $NaBH_4$ (ca 30 mg) added twice at 20 min intervals, as the intervals of 10 min used by Testa & Beckett (1972) did not always show complete reduction. The buffer used by Hossie (1970), and by Testa & Beckett (1972) was also omitted, as the same results were obtained with or without it.

Table 2 gives results obtained with the new method for the analysis of urine samples collected after administration of the diethylpropion.

Examples of the excretion curves of diethylpropion and its metabolites under acidic urine conditions of one of the subjects used are shown in Figs 4, 6. As may be seen, the metabolism of diethylpropion after oral administration is complex. The total recovery of diethylpropion and its metabolites (Table 2) accounted for about 70% of the dose; about 30% of the drug, which was not detected in the form of basic metabolites, probably occurs as deaminated product(s) through phenylmethyldiketone to benzoic acid, which is predominantly conjugated with glycine to give hippuric acid (Scheme) (e.g. see Schreiber et al 1968). This is also in agreement with findings of Dring et al (1966) for amphetamine. Less probable is the formation of hydroxylamines and their partial excretion in conjugated forms, since it is considered that even if hydroxylamines are formed metabolically they are reduced in-vivo to the parent amine (Coutts & Beckett 1977).

The low recovery of the unchanged drug, together with a low peak level and a short observed half-life, strongly support the supposition that diethylpropion itself contributes very little to the total pharmacological activity observed. This also confirms the hypothesis of Jasinski et al (1974) and Garattini et al (1978) that a hepatic metabolite is the active moiety, as diethylpropion is consistently more potent by the

Table 2. The recoveries in man of diethylpropion and its basic metabolites excreted under acidic urine conditions after oral administration of 25 mg of the hydrochloride in aqueous solution, or of 75 mg of the hydrochloride in sustained release pellet formulation (SRF, lot 22-A-78).

	The recoveries of compounds I to VI, expressed as percentages of the dose*					
	25 mg of diethylpropion HCl			75 mg of diethylpropion HCl (SRF)		
Compounds recovered‡	Subj. 1,a†	Subj. 1,b	Subj. 2	Subj. 1	Subj. 2	Subj. 3
I	6·6	4·5	3·2	3·9	2·9	3·7
II	32·9	36·7	36·4	31·0	39·2	22·1
IV	20·1	17·3	13·4	25·5	16·7	16·7
V	3·8	2·6	3·8	3·3	5·0	4·1
III + VI	8·2	9·6	14·1	7·4	15·7	12·3
Duration	25 h	26 h	14 h	48 h	45 h	48 h
Total recovery	71·6	70·7	70·9	71·1	79·5	58·9
Total of dealkylated compounds (II, III, V and VI)	44·9	48·9	54·3	41·7	59·9	38·5
Total of reduced compounds (IV and V)§	23·9	19·9	17·2	28·8	21·7	20·8

* The percentage of the dose is calculated from the total excreted amount of each compound as the equivalent amount of compound I.
† Drug was taken after meal.
‡ I = Diethylpropion, II = *N*-ethylaminopropiophenone, IV = *N*-diethylnorephedrine, V = *N*-ethylnorephedrine, III = aminopropiophenone, VI = norephedrine.
§ Contribution of VI is considered to be very low.

oral than by the subcutaneous route and showed discrepancies between in-vitro and in-vivo effects.

N-Dealkylation (compounds II, III, V and VI) products account for 45–55% of the dose, and reduction (compounds IV, V and VI) accounts for about 20–25% of the dose (Table 2). Metabolite VI was not determined separately, but its contribution to the total quantity of reduced metabolites is considered to be in the range of the variations observed, i.e. 1–5%. This shows *N*-de-ethylation, particularly mono-*N*-de-ethylation as the most important pathway in the metabolism of diethylpropion. This has not been shown previously, mainly because of the incomplete and inadequate analytical procedures used (Schreiber et al 1968; Banci et al 1971; Testa & Beckett 1972; Wright et al 1975).

The differences between the present work on diethylpropion and that of Testa & Beckett (1972) may be summarized as follows.

(i) Some higher recoveries of unchanged drug were found in the present work. The reason could be that in the earlier work metabolite IV + the decomposition product of I were subtracted from the peak height ratio of compounds I + IV obtained after reduction, or that reduction carried out at 2 × 10 min intervals was not quantitative.

(ii) Higher concentrations of metabolite II were obtained in the present work because in the earlier work the peak height ratio of V and the decomposition product of metabolite II were subtracted from the peak height ratio of II + V (as V).

(iii) Regular excretion of metabolite III was shown in the present work because in the earlier work subtraction of the peak height ratio obtained before reduction, from the peak height ratio obtained after reduction, was possible only when reduction was not complete (see Discussion).

(iv) Quantities of metabolite IV corresponded well with those reported in previous studies due to the fact that the decomposition product of I, as I was present in low concentrations, had a negligible effect on the peak height of IV. Thus, the failure to take it into account in previous studies did not result in a significantly altered concentration of compound IV.

(v) Metabolite V was present to a lesser amount in the present work, because in the previous work metabolite V was measured together with the decomposition product of metabolite II.

(vi) Metabolites III + VI were found herein in the range of about 1–5%, while about 30% of the dose was stated to be present by Testa & Beckett (1973, 1974), because co-extracted metabolite II was measured as metabolite VI.

(vii) Consequently, the total recoveries of diethylpropion and its basic metabolites found in this work were about 20% lower than those obtained in the previous work.

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