

# In vivo Screening of Glutathione Related Detoxification Products in the Early State of Drug Development

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*Dedicated to Professor Helmut Simon on the occasion of his 60th birthday*

Drug-Conjugates, Glutathione, Screening, HPLC

Glutathione (GSH) adducts and consecutive degradation products thereof are indications of reactive intermediates during drug metabolism. As demonstrated with the analgesic SX-PP 16 (4-amino-3,5-dibromacetanilide), however, interactions of a drug with GSH can be detected by labelling the GSH-stores with labelled cysteine, and consecutive administration of the unlabelled drug even at therapeutic doses. The GSH-adducts are sensitively and specifically traced by HPLC, applying column-switching and a combination of diode array- and radioactivity detection. This approach seems to be much more sensitive than a classical GSH-depletion study. The structure of the main metabolite of SX-PP 16 (46% of urinary excretion) was elucidated as 3-bromo-4-amino-5-mercaptopuryl-acetanilid.

## Introduction

Glutathion (GSH) conjugates, and consecutive degradation products thereof, are severe indications of reactive intermediates during drug metabolism [1, 2]. The unambiguous detection of such reactives would be of great importance during early stages of drug development. The normally applied GSH-depletion measurements, however, suffer from their poor sensitivity. This is valid especially as low drug doses are concerned, since the measurement of a small decrease from a huge GSH store is involved. Therefore the rational approach is to trace the products of GSH interaction.

The isolation of such sulfur containing metabolites, however, is difficult and time consuming and therefore unsuitable for a screening method.

However, a specific and sensitive approach for tracing GSH conjugates and their degradation products can be performed by co-administration of unlabelled drug and radioactive cysteine. The latter labels the GSH stores and related pathways in the organism [3, 4]. The specific and sensitive detection of such drug related products can then be conveniently established by application of modern HPLC techniques.

To find out scope and limitations, this approach was applied to the analgesic SX-PP 16. Since there

are similarities to acetaminophen, its interaction with GSH was of superior interest. In addition, the investigation of SX-PP 16 was stimulated by the discrepancy between the results of classical GSH depletion studies and structure elucidation of its main biotransformation products.

## Material and Methods

### The drug

SX-PP 16 (4-amino-3,5-dibromacetanilide) acts as an analgesic drug.

We used non-labelled and the  $^{14}\text{C}$ -labelled SX-PP 16  $1.49 \times 10^8$  Bq/mmol, uniformly labelled in the aromatic ring (Purity > 99%).

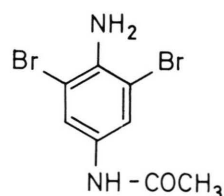


Fig. 1. SX-PP 16, an analgesic drug under research.

### Labelled cysteine

L- $^{35}\text{S}$ ]Cysteine, 11.1 TBq/mmol, NEG-020. Purity > 96%. NEN-Research.

L- $^{14}\text{C}$ ]Cysteine, 1.1 GBq/mmol, CFB 108. Purity > 90%. Amersham Buchler.

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### Animals and dosing

For the studies male rats of 200 g body weight Chbb/Thom were used. Each animal received 100  $\mu$ Ci labelled cysteine orally by gavage as an oxygen-free solution together with about 20 mg unlabelled cysteine. For the application of the drug a slurry of SX-PP 16 in 1% of Tylose<sup>R</sup> SL 400 was prepared in a concentration range of 5 to 30 mg/kg. The final application volume was in each case 1 ml. For collection of bile we used conscious (p.o.-dosing) and anesthetized (i.d.-dosing) animals.

The bile collection gadget used in conscious animals was prepared according to the method described by Tomlinson [5].

### Instruments

#### Mass spectrometry

The EI mass spectra (70 eV) were recorded with a Kratos AEI MS 902 updated by VG Instruments (Manchester, GB) which was linked to an Incos data system (Finnigan, San Jose, USA). The samples were introduced *via* a direct inlet system and evaporated by heating the source to 120–150 °C. The FAB mass spectra were performed using the same instrument, provided with a VG FAB ion source of standard design. The samples were generally dissolved in 1–3  $\mu$ l methanol and mixed with 3–5  $\mu$ l glycerol on top of the stainless steel target to form a clear solution. The target was inserted into the ion source by a direct inlet system and bombarded with a 8–10 keV beam of Xenon atoms. The generated ions were extracted from the source and accelerated with 8 kV. The mass spectra were taken with scan rates of 10 or 15 sec/decade at a resolution of approximately 2000 (10% valley definition).

#### NMR spectroscopy

The <sup>1</sup>H NMR spectra were recorded in pulse Fourier transform mode using 80, and 400 MHz spectrometers of Bruker (Karlsruhe, FRG).

Deuteriochloroform, d<sub>4</sub>-methanol and mixtures thereof served as solvents, whereas tetramethylsilane was used as an internal standard.

#### HPLC

Pumps: a) Varian 5060 with ternary gradient (Walnut Creek, USA). b) Gyncolec isocratic pump (Munich, FRG).

UV: Hewlett Packard 1040 A (Palo Alto, USA).

Radioactivity: Isomess Ramona D (Straubenhard, FRG) with Wang or Apple Computer.

Column: Knauer 250 × 8 mm with 5  $\mu$ m ODS (Berlin, FRG).

Flow: 2 ml/min.

#### Gradientprofile

Time [min]	Solvent (vol %)	
	A %	B %
0	90	10
2	90	10
25	60	40
30	60	40
35	10	90
38	10	90
42	90	10

A = water / 0.5% acetic acid.

B = methanol.

The HPLC was used in two column-switching arrangements.

A) The arrangement without quantifying the extraction yield was built according to the equipment described by Roth [6].

B) The fully automated equipment was built according to Schmid [7]. It allows the quantification of the extraction yield. Briefly, the urine is loaded onto a precolumn, the precolumn is flushed with water containing 1% ammoniumacetat for 6 min. This effluent is monitored by radioactivity detection. After 6 min the content of the precolumn is transferred to the main column and the gradient program is started. The radioactivity detector is switched from the precolumn to the main column to monitor the separation.

For profiling of body fluids 2 ml of urine or 0.5 ml of bile were injected onto the HPLC precolumn.

#### Isolation of the main metabolite

The urine of the 0–24 h collection period (176 ml) was extracted with 12 g XAD-4, Röhm and Haas (Darmstadt, FRG). After washing with 240 ml water, the XAD-4 was eluted with 370 ml methanol.

The organic phase was evaporated under reduced pressure. The residue was redissolved in 40 ml water. This phase contained 92.1% of the original radioactivity and was separated in 15 runs with the gradient described above. The separated compounds were collected. The main metabolite was further ap-

plied as a band to a TLC silica-acid sheet, Merck No. 5714 (Darmstadt, FRG) 20×20 cm and developed with a mixture of chloroform, ethanol, acetic acid 70:25:5 (v/v). The main metabolite had a relative retention of 0.24.

The band of interest was scrapped with a spatula and the compound eluted with methanol/water 90:10. The extract was finally chromatographed on the HPLC system described above, however without acetic acid.

## Results

### Glutathione depletion study

The results of a classical GSH depletion study are depicted in Table I. SX-PP 16 has only a moderate influence on the depletion of GSH-stores.

Table I. Glutathione depletion. Rats were dosed 2 h before the analysis of the organ with different doses of SX-PP 16. The GSH levels were determined according to Ellman [8].

Dose [mg/kg]	n	mmol GSH/ g organ	SD	Reduction
Control	8	7.5	0.78	—
300	7	5.9	0.50	21%
600	8	5.3	0.66	30%
1200	8	5.2	0.46	31%

n = Number of animals.

### Incorporation of labelled cysteine into the SX-PP 16 molecule by biotransformation

The pattern of radioactivity in urine with 100  $\mu$ Ci [ $^{14}$ C]cysteine alone and with co-administration of 30 mg/kg SX-PP 16 revealed additional peaks in the HPLC chromatogram at about 28–32 min (Fig. 2). The peaks at a retention time of 3–8 min are apparently due to endogenous reaction products of the administered cysteine (presumable taurine).

The result obtained with bile differs (Fig. 3), as there are not only peaks at 2–7 min in the blanc but also a major group of peaks at 37–42 min.

However, the group of peaks at a retention time of 26–32 min is related to the co-administered drug. Rather the same pattern is observed when [ $^{35}$ S]cysteine (100  $\mu$ Ci) and 30 mg SX-PP 16 is dosed (Fig. 4). The individual pattern in bile is rather simi-

lar in all five animals. The percentage of radioactivity excreted during the 2–8 h period was 30.5%  $\pm$  6.60% (n = 5) of the dosed radioactivity.

On the other hand the pattern in urine is consistent in 4 of 5 animals. The percentage of radioactivity excreted during the 2–8 h period was 10.2%  $\pm$  0.53%. One animal with low excretion during this period caught up with the others during the following 8–26 h collection interval. The first peaks between 3 and 8 min show very poor retention behaviour on the precolumn. They cannot be used for interpretation. This problem could be overcome, however, by changing the precolumn switching mode (HPLC method B, Materials and Methods).

### Improving of the detection limit

Low doses of the drug in question or formation of the corresponding GSH adducts in low yields only, give rise to problems concerning the radioactivity

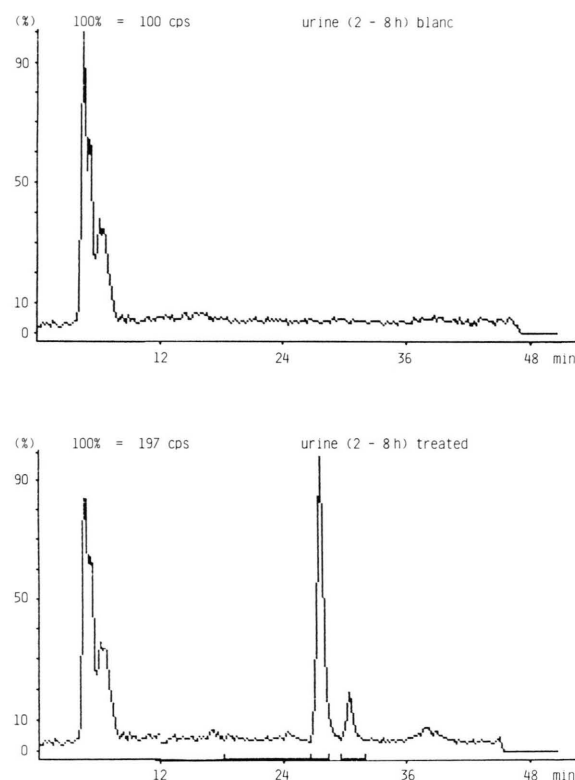


Fig. 2. Urinary pattern of radioactivity after dosing of 100  $\mu$ Ci [ $^{14}$ C]cysteine without (= blanc) and with oral co-administration of 30 mg/kg (= treated) SX-PP 16 (HPLC method A).

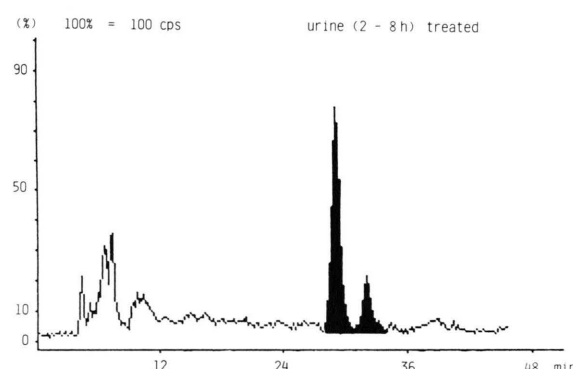
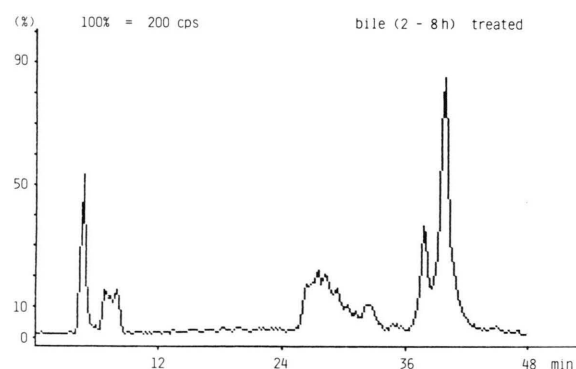
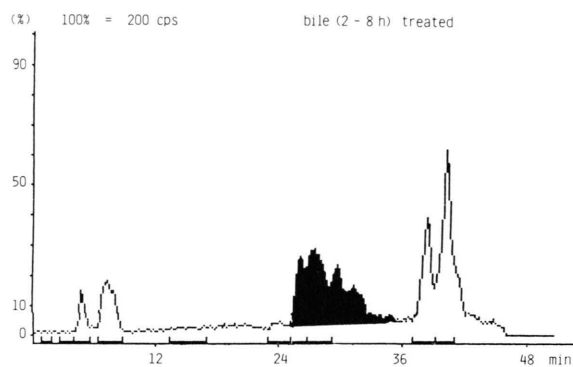
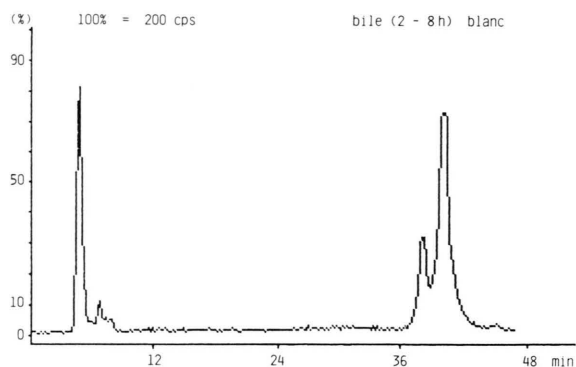


Fig. 3. Biliary pattern of radioactivity after dosing of 100  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]cysteine without (= blanc) and with i.d.-co-administration of 30 mg/kg (= treated) SX-PP 16 (HPLC method A).

Fig. 4. Pattern of bile and urine in conscious rats: 100  $\mu\text{Ci}$  [ $^{35}\text{S}$ ]cysteine and 2 h later 30 mg/kg SX-PP 16 were applied. The percentage of dose in this fraction is indicated. The peaks which were not in the blanks (without drug administration) are coloured black (HPLC method A).

and further identification of these products. This can be overcome by simultaneous diode array detection of the corresponding drug chromophor using the technique of isoabsorbance plots [17].

In Fig. 5 a HPLC chromatogram of rat bile is depicted which was collected 2–8 h after dosing 50  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]cysteine and 5 mg/kg SX-PP 16 with the result of a low yield of GSH conjugates.

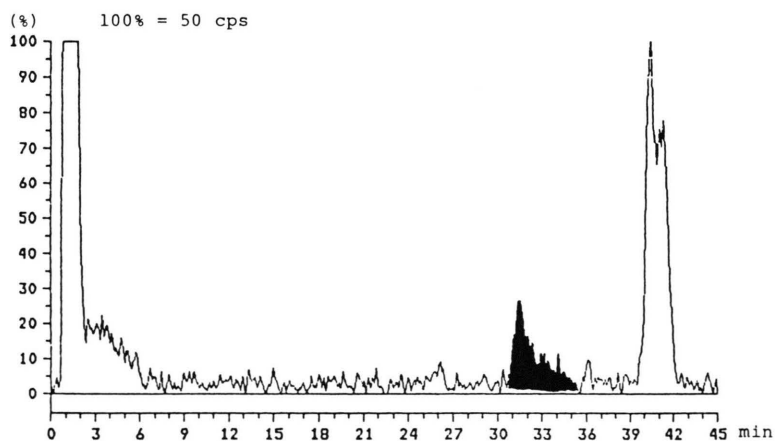


Fig. 5. HPLC chromatogram (HPLC method B) of rat bile 2–8 h after dosing 50  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]cysteine and i.d.-administration of only 5 mg/kg SX-PP 16, thus with low yield of GSH conjugate.



The principle of the isoabsorbance plot is illustrated in Fig. 6. It shows the UV spectrum of SX-PP 16 together with the corresponding isoabsorbance plot obtained during an HPLC run.

The UV spectrum of SX-PP 16 is characterized by three maxima: 215 nm, 262 nm, 314 nm. The absorption maxima at longer wavelengths are more relevant for compound identification.

According to this technique the diode array trace of the retention region between 30–43 min is depicted in Fig. 7. At approximately 31.5 min there is a signal as well in the UV plot as in the radioactivity traced chromatogram showing a typical picture of three maximas (220 nm, 262 nm and 320 nm). Thus the peak at 31.5 min should contain the chromophore of SX-PP 16. In contrast at a retention time of 40–43 min there is no indication for such an UV spectrum. Thus only the peak at 31.5 min should be

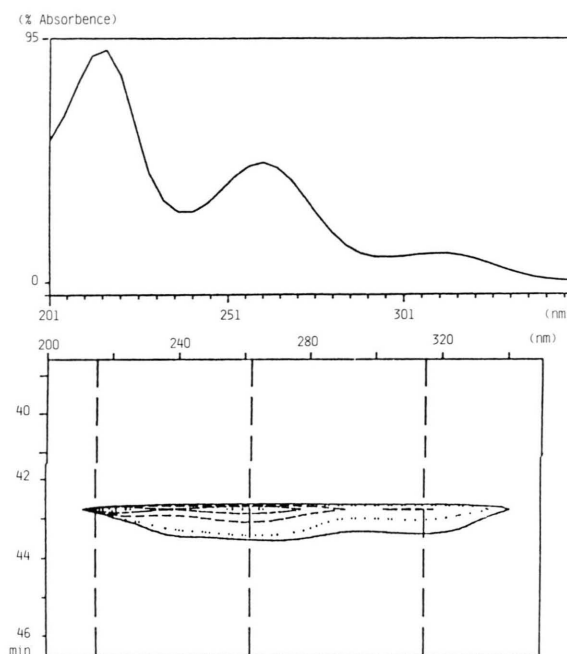


Fig. 6. UV spectrum and isoabsorbance plot of a HPLC peak of the parent compound SX-PP 16.

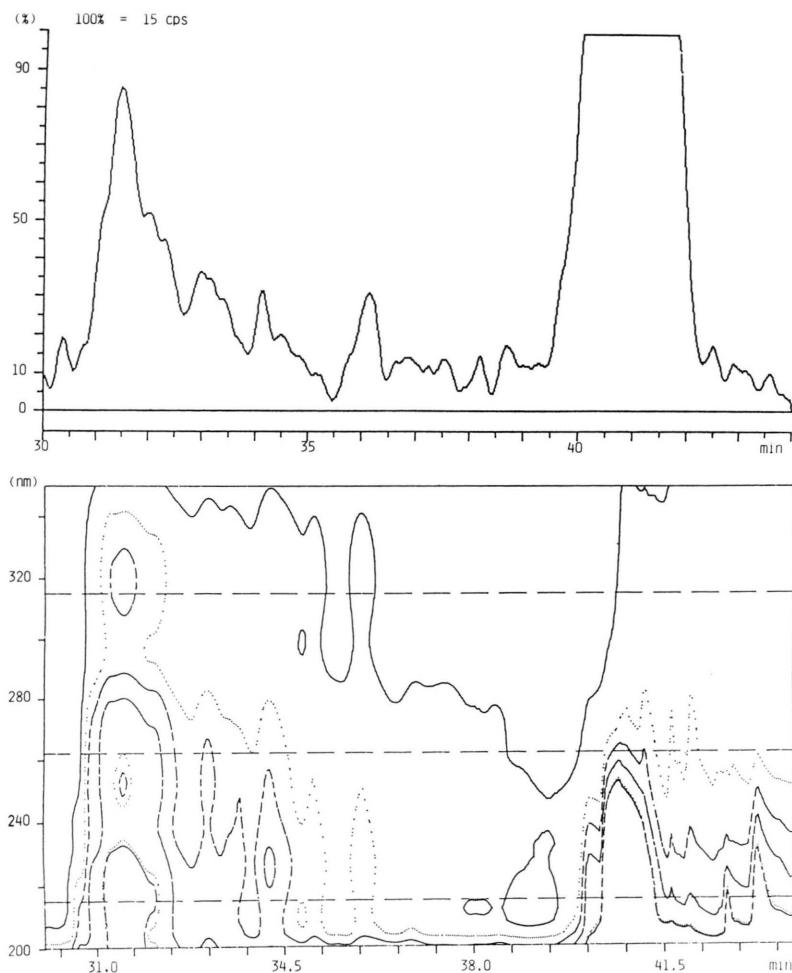


Fig. 7. Top: Part of the HPLC chromatogram of Fig. 5. Bottom: Isoabsorbance plot of the diode-array detection of the same retention region. The dotted lines at 215 nm, 262 nm, 314 nm indicate the absorption maxima of the parent compound.

related to S-conjugates of SX-PP 16 but not the peaks between 40–43 min.

#### Timing of cysteine and drug dosage

To evaluate the optimal timing of labelled cysteine and drug the following experiment was performed:

Rats received at zero time 100  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]cysteine and 2 h later 30 mg/kg SX-PP 16. At the second and third day again a dose of 30 mg/kg SX-PP 16 was administered but without predosing labelled cysteine. The results are shown in Fig. 8. Thus the best sampling time providing the highest yield of labelled GSH conjugates is the time interval 2–26 h after application of labelled cysteine.

#### Isolation and structure elucidation of the main metabolite

To get a brief insight into the metabolic pattern of  $^{14}\text{C}$ -labelled SX-PP 16 we focused our attention to

urine and bile only. At this point of investigation there was no need to perform a complete balance study including feces.

For the isolation of the metabolites 30 mg  $^{14}\text{C}$ -labelled SX-PP 16 was administered to 5 animals. The urinary and biliary excretion is summarized in Table II.

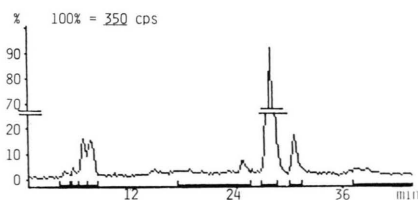
Fig. 9 (top) represents the urinary pattern of the collection period 0–24 h p.a. The radioactivity in the retention range of 1–6 min is caused by not retained metabolites.

In Fig. 9 (bottom) the biliary pattern of the collection period 0–8 h p.a. is depicted.

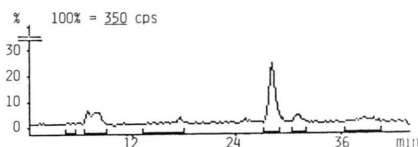
The following results are apparent: There are only 15.4% of the parent compound (MO) in the urine and 4.3% in the bile. The metabolite M12 is the main metabolite in *urine*. It represents 46% of the excreted radioactivity. This metabolite is also present as the main peak in the metabolic pat-

#### 1st day

time 0h: 100  $\mu\text{Ci}$   $^{14}\text{C}$ -cysteine  
time 2h: 30 mg/kg SX-PP16  
Urine 2–8h →

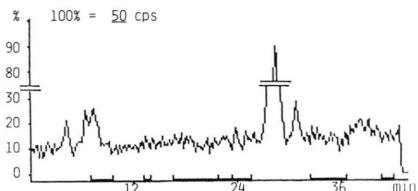


time 8h: -  
Urine 8–26h →

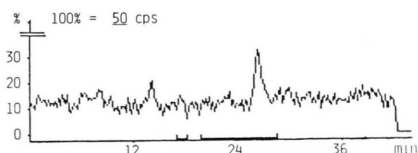


#### 2nd day

time 26h: 30 mg/kg SX-PP16  
Urine 26–34h →



time 34h: -  
Urine 34–50h →



#### 3rd day

time 50h: 30 mg/kg SX-PP16  
Urine 50–58h →

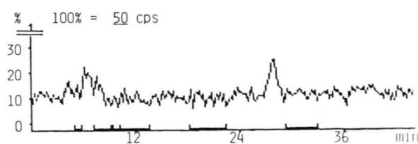


Fig. 8. Timing of the administration of cysteine and drug.

Table II. Urinary and biliary excretion of radioactivity after dosing 30 mg/kg [ $^{14}\text{C}$ ]SX-PP 16.

	Time	<i>n</i>	<i>x</i> [%]	SD [%]
Urine	0–8 h	3	4.8	
	8–24 h	3	27.5	
	24–48 h	3	12.5	
	0–48 h		43.8	5.20
Bile	0–8 h	3	17.0	5.38

*n* = Number of animals.*x* = Percentage of total applied radioactivity.

tern when labelled cysteine together with unlabelled SX-PP 16 is administered. This is also valid for the bile metabolites.

After isolation and derivatization with diazomethane, structure elucidation of the main

metabolite M12 could be performed by its EI and FAB mass spectra and by its  $^1\text{H}$  NMR spectrum depicted in Fig. 10–12. The EI mass spectrum in Fig. 10 shows a very weak molecular ion at  $m/z$  403/5 according to the presence of one bromine atom only. Its further breakdown which is also schematically outlined in Fig. 10 corresponds to that of 3-mercaptopuryl-acetaminophene [20]. FAB mass spectrometry confirmed the suggested mercapturic acid methyl-ester in the abundant ( $M+H$ ) ion  $m/z$  404/6. The intense ion  $m/z$  144 represents the structural moiety of the N-acetylated amino acid methylester as indicated in Fig. 11.

The proposed structure of a 3-bromo-4-amino-5-mercaptopuryl acetanilid for the metabolite M12 is fully established by the  $^1\text{H}$  NMR spectrum in Fig. 12. The *meta* position of the two aromatic protons is indicated by the doublets ( $j=3$  Hz) at 7.41 and 7.71 ppm respectively (Fig. 12, bottom).

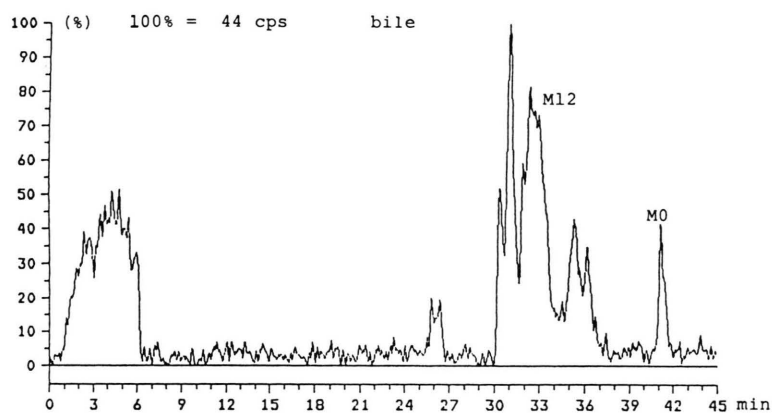
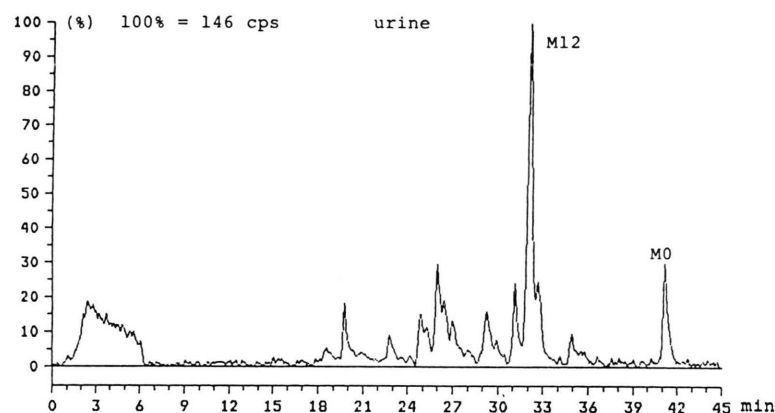
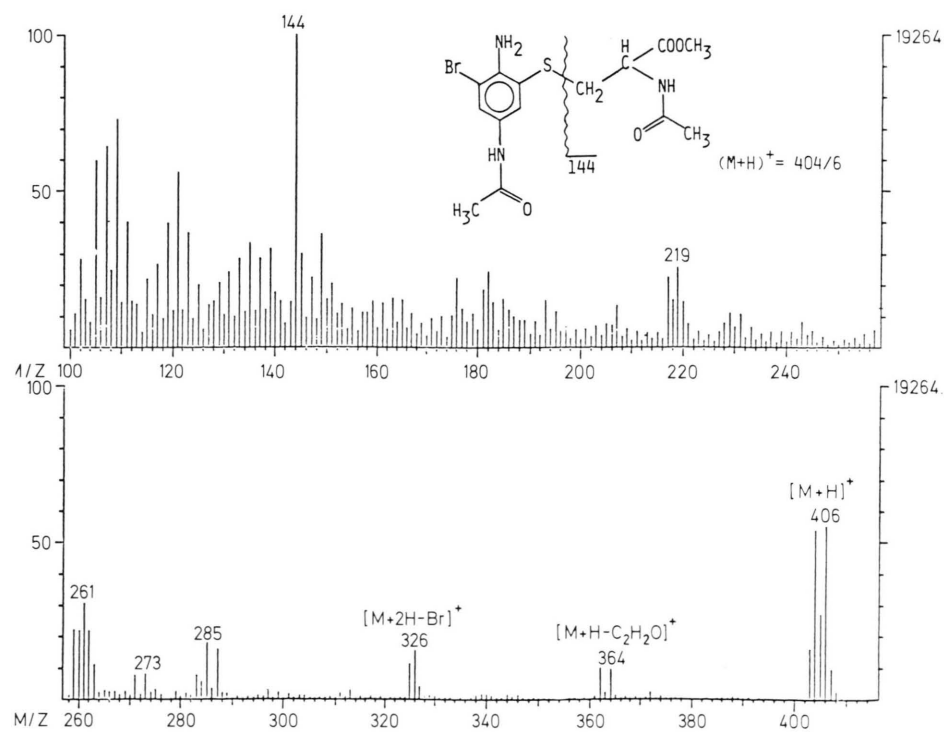
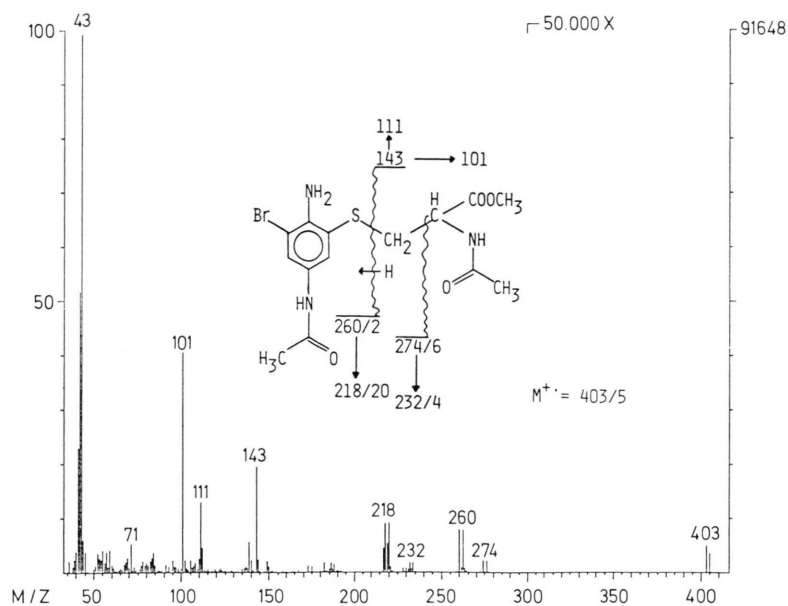


Fig. 9. Top: Metabolic pattern in urine (0–24 h p. a.) of rats dosed orally with 30 mg/kg [ $^{14}\text{C}$ ]SX-PP 16 (HPLC method B). Bottom: Metabolic pattern in bile (0–8 h p. a.) of rats dosed i.d. with 30 mg/kg [ $^{14}\text{C}$ ]SX-PP 16. M12 = main metabolite, MO = parent compound.



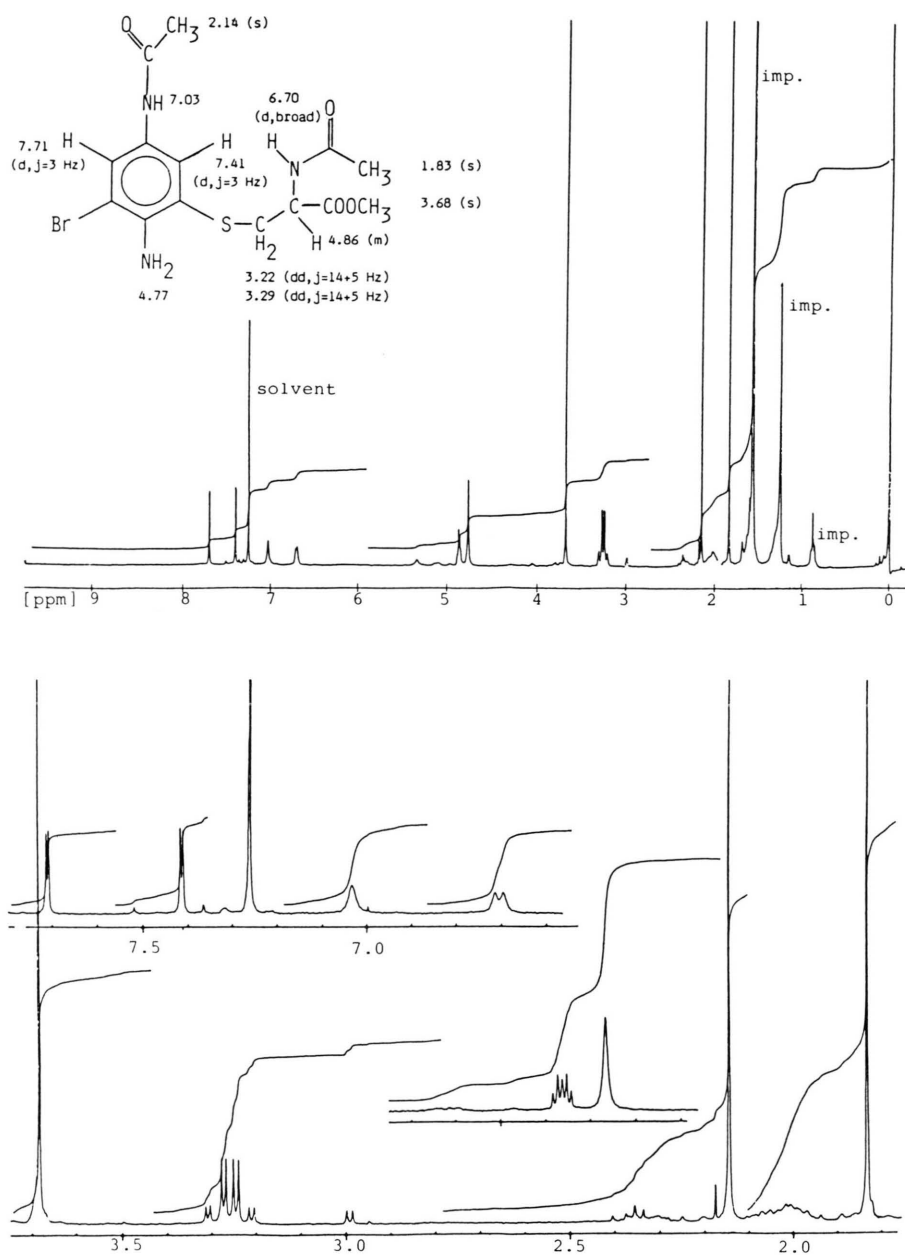


Fig. 12. Top: <sup>1</sup>H NMR spectrum of the main metabolite M12 in CDCl<sub>3</sub> (methyl ester derivative). Bottom: Expanded parts of the <sup>1</sup>H NMR spectrum of metabolite M12.

## Discussion

Methods which should be routinely used during pharmacokinetic and metabolic studies must be sensitive, specific and convenient. At early stages of drug development an additional handicap arises in so far as these drugs are not available as a labelled compound.

The interaction of a drug with GSH is mostly studied by its influence on the GSH levels of organs by colorimetric [8] or electrochemical [19] procedures. These methods measure the *decrease* of the huge GSH stores and not the *formation* of drug GSH adducts.



Our suggested approach for the specific and sensitive detection of GSH conjugates and their degradation products by labelling the GSH stores in the organism, and administration of the unlabelled drug in therapeutical doses, has been convincingly performed with the analgesic drug SX-PP 16. Thus, the pattern of radioactivity in urin of rats receiving 100  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]cysteine alone or with co-administration of 30 mg/kg unlabelled SX-PP 16 revealed unambiguously additional peaks by comparison of the corresponding HPLC chromatograms (Fig. 2). The same result could be obtained with bile even though there are two groups of peaks in the HPLC chromatograms which are related to endogenous compounds (Fig. 3). Both results could be verified by feeding the animals with 100  $\mu\text{Ci}$  [ $^{35}\text{S}$ ]cysteine (Fig. 4) indicating that there are no substantial differences between [ $^{14}\text{C}$ ]- and [ $^{35}\text{S}$ ]cysteine in this case. However, using [ $^{35}\text{S}$ ]cysteine all sulfur containing metabolites of the drug can be detected.

Taking a time interval between dosing of radioactive cysteine and the drug respectively, the possibility arises of collecting urine and bile before drug administration. This means that an individual blanc can be obtained from each animal. As shown in Fig. 8 administration of SX-PP 16 2 h after cystein dosage still gives reasonable yields of drug conjugates in urine, collected in intervals of 2–8 and 8–26 h p. a. respectively. However, additional doses of SX-PP 16 one or two days later do not enhance the yields. This is in agreement with the observation of Lauterburg [4] and Tateishi [15], who determined the hepatic GSH turn-over of the main pool to be about 1.5 h. The half-life of radioactivity excretion by urine is determined not only by the formation rate of the drug conjugates but also by their elimination characteristics. In so far, the excretion of conjugate at late collection intervals can not be primarily attributed to the second GSH pool according to Tateishi [15].

Principally it is a probleme to detect unknown structures with unknown chromatographic and spectroscopic behaviour. However, this can be overcome making use of the potencies of modern HPLC. From our experience with SX-PP 16 and other drugs a reversed phase C18 column with a gradient, ranging from acidic water to methanol is a reasonable choice to trace most of the metabolites like mercapturic acids generated by reaction of basic drugs with GSH. Very polar metabolites in urine and bile which are not retained on the precolumn, however, should be

quantified too. The column switching unit (method B) allows to monitor the not retained fraction, and thus to quantify the extraction yield achieved by the HPLC precolumn automatically.

The sensitivity of the method is limited by the HPLC detection, since it is based on the differences between HPLC chromatograms of drug untreated (blanc) and treated animals (Fig. 2). The coincidence of the radio signal and the drug characteristic UV signal (in our case 262 and 320 nm) enhances the probability of correct identification considerably since a GSH conjugation usually does not change the chromophor of a drug drastically. If there are further questions about the identity of the peak it might be collected and chromatographed in a different solvent system to a constant ratio of UV *versus* radioactivity signal.

Comparing the metabolic pattern regarded to the application of the  $^{14}\text{C}$ -labelled SX-PP 16 and unlabelled drug together with labelled cysteine respectively there is strong evidence that most of the metabolites of SX-PP 16 are formed by reaction of the drug with GSH. As the investigation was not performed to be a complete biotransformation study of this drug, we focused our attention on the main metabolite in urine. All our data are consistent with the structure of a mercapturic acid formed by substitution of one bromine atom (Fig. 12).

The biological insertion of a sulfur containing moiety (above all GSH) into a drug is the result from nucleophilic attack at an electron deficient center [3, 9, 10, 16].

In the case of SX-PP 16 the formation of the 5-mercapturic acid is thought to proceed *via* an electrophilic quinonimine intermediate. This is possibly generated by oxidation of the parent compound by cytochrome P-450/P-448 mediated monooxygenases.

This pathway is of particular interest, as the structural related quinonimines originated from 3,5-dimethylacetaminophen and the 2,6-dimethyl-isomer were studied with respect to GSH interaction [11, 18]. The 3,5-dimethyl compound (in contrast to the 2,6-isomer) did not produce any GSH adduct. The different behaviour of SX-PP 16 is attributed to the stabilization of a presumable formed GSH adduct by elimination of a bromide anion. On the other hand, substitution of aromatic bonded halogens by sulfur-moieties are not unusual, *e.g.* the chemicals chloronitrobenzenes, hexachlorobenzene [12], the drugs flumazenil [13] and a pyrano-quinoline [14]. The

outstanding high yield, however, in the case of SX-PP 16 is unexpected and remarkable.

All our findings concerning the incorporation of labelled cysteine into the SX-PP 16 molecule are in contrast to the results obtained by a classical GSH depletion study according to Ellmann [8]. We could detect only a moderate effect of SX-PP 16 on the GSH levels even at doses 10–40 times higher (Table I). These results mirror the general difficulties of all approaches taking changes of GSH levels in the organs of certain species into account. Since the turnover of GSH is comparable short (approximately 1–2 h) and different in different species the timing of the depletion measurements become ambiguous regarding the time of drug application. In addition this situation is complicated by the fact that nothing is known about pharmacokinetics and disposition of the drug at this state of development. However, the complexity of such investigations is elegantly overcome by the offered method of labelling the GSH stores. As far as SX-PP 16 is concerned there might be two additional explanations on hand for the only moderate depletion of GSH levels:

- i The pathway yielding a quinonimine is mediated by enzymes which are saturable. In the case of

SX-PP 16 there are indications that the cytochromes P-448 subclass is involved in the biotransformation of this compound. It is well-known that P-448 have a low contribution to total cytochrome content in non-induced rats.

- ii The absorption of SX-PP 16 might be incomplete at higher dosages as SX-PP 16 is rather insoluble in all application formulations tested so far.

In conclusion, we have shown that a drug/GSH interaction can be conveniently detected by labelling the GSH stores with labelled cysteine and subsequent tracing of the formed conjugates by modern HPLC techniques. Apparently this approach has the following advantages over a classical GSH depletion study: There is no ambiguity regarded to the timing of the experiments. It is absolutely specific and more sensitive and it even works at therapeutic doses. Furthermore there are less animals needed and each animal can be used as its own control.

#### Acknowledgement

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- [1] E. Boyland and P. Sims, *Biochem. J.* **68**, 440–446 (1958).
- [2] B. Ketterer, *Xenobiotica* **16**, 957–973 (1986).
- [3] A. Warrander, J. M. Allen, and R. S. Andrews, *Xenobiotica* **15**, 891–897 (1985).
- [4] B. H. Lauterburg, Y. Vaishnav, W. G. Stillwell, and J. R. Mitchell, *J. Pharmacol. Exp. Ther.* **213**, 54–58 (1980).
- [5] P. W. Tomlinson, D. J. Jeffery, and C. W. Filer, *Xenobiotica* **11**, 863–870 (1981).
- [6] W. Roth, K. Beschke, R. Jauch, A. Zimmer, and F. W. Koss, *J. Chromatogr.* **222**, 13–22 (1981).
- [7] J. Schmid and W. Roth, under publication in: *Proceedings of the 9th European Drug Metabolism Workshop* (D. Benford, ed.), Guildford, Taylor and Francis, London 1986.
- [8] G. L. Ellmann, *Arch. Biochem. Biophys.* **82**, 70–77 (1959).
- [9] L. J. Fischer, M. D. Green, and A. W. Harman, *Drug Metabol. Disp.* **13**, 121–126 (1985).
- [10] M. Tateishi, *Drug Metabol. Reviews* **14**, 1207–1234 (1983).
- [11] G. M. Rosen, E. J. Rauckman, S. P. Ellington, D. C. Dahlin, J. L. Christie, and S. D. Nelson, *Molec. Pharmacol.* **25**, 151–157 (1984).
- [12] L. F. Chasseaud, in: *Glutathione, Proceedings of the 16th Conference of the German Soc. of Biol. Chem.* Tübingen 1973 (L. Flohé, ed.), pp. 90–109, Georg Thieme, Stuttgart 1974.
- [13] H. R. Sullivan and R. B. Franklin, *Drug Metabol. Disp.* **13**, 276–278 (1985).
- [14] D. A. Smith, P. M. Johnson, and D. J. Wilkinson, *Xenobiotica* **15**, 437–444 (1985).
- [15] N. Tateishi and T. Higashi, in: *Functions of Glutathione in Liver and Kidney* (H. Sies, ed.), pp. 3–21, Springer, Berlin 1978.
- [16] S. Orrhenius and P. Moldeus, *Trends Pharmacol. Sci.* **1984**, 432–435.
- [17] A. F. Fell, B. J. Clark, and H. P. Scott, *J. Pharm. Biomed. Anal.* **1**, 557–572 (1985).
- [18] R. van de Straat, J. de Vries, T. Kulkens, A. J. J. Debets, and N. P. E. Vermeulen, *Biochem. Pharmacol.* **35**, 3693–3699 (1986).
- [19] C. B. Jensen, S. J. Grossman, and D. J. Jollow, in: *Biological Reactive Intermediates III* (J. J. Kocsis, ed.), pp. 407–413, Plenum, New York 1986.
- [20] S. D. Nelson, Y. Vaishnav, H. Kambara, and T. A. Baillie, *Biomed. Mass Spectrom.* **8**, 244–251 (1981).