*Journal of Chromatography*, 573 (1992) 150–153
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6099

# Short Communication

# Evaluation of 2-(2-thiophenecarboxy)benzoic acid and related active metabolites in biological samples

C. Lucarelli

*Istituto Superiore di Sanità, Viale Regina Elena 199, 00161 Rome (Italy)*

R. Pelloso, G. Bruno* and C. La Rosa

*Medea Research, Via Pisacane 34/A, 20100 Milan (Italy)*

F. Belliardo

*Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, 10100 Turin (Italy)*

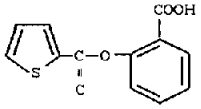
(First received May 22nd, 1991; revised manuscript received July 15th, 1991)

ABSTRACT

This paper describes a quantitative assay of 2-(2-thiophenecarboxy)benzoic acid and its systemic metabolites, namely salicylic acid and thiophenecarboxylic acid, by high-performance liquid chromatography with ultraviolet detection at 254 nm. Analytes were extracted from acidified samples with *tert.*-butylmethyl ether and separated with an RP-18 column (150 mm × 3 mm I.D., 5 μm particle size) with a mixture of 0.01 *M* potassium phosphate and methanol (70:30, v/v) at pH 3.1. The method proved to have the validation required for pharmacokinetic investigations in animals and humans.

## INTRODUCTION

2-(2-Thiophenecarboxyl)benzoic acid (YS 134, TBA) is a new interesting compound obtained by esterifying salicylic acid (SA) with 2-thiophenecarboxylic acid (TA) (Fig. 1). TBA is endowed with anti-inflammatory, analgesic and antipyretic properties, possessing a gastric tolerability better than is usually the case with non-steroidal anti-inflammatory agents (NSAIAs) and acetylsalicylic acid (ASA), as previously described for other NSAIAs in esterified form [1–5]. Preliminary pharmacokinetic studies in rats show that after oral administration TBA is transformed into SA and TA.




$C_{12}H_8O_4S$
M. W. = 248.26

Fig. 1. Structure of TBA.

This paper describes an analytical method validated to evaluate TBA and its two main systemic metabolites in biological samples.

0378-4347/92/$05.00 

## EXPERIMENTAL

### *Chemicals and instruments*

YS 134 was kindly supplied by Medea Research (Milan, Italy). All solvents and chemicals used were of analytical or high-performance liquid chromatography (HPLC) grade. Working standards of TBA, SA, ASA, TA and naproxen were carefully checked for chemical identity and purity.

A liquid chromatograph equipped with a UV detector was used for the analysis. The column was RP-18 (glass column), 5 $\mu$m particle size, 150 mm × 3 mm I.D. Evaluations were carried out using a personal computer with adequate software for statistical and pharmacokinetic analysis.

### *Extraction and chromatographic conditions*

A 1-ml aliquot of plasma (or urine or tissue homogenate) was placed into a glass-stoppered test tube with 20 $\mu$g of naproxen as internal standard, acidified with 1 ml of 0.1 *M* hydrochloric acid and extracted with 2 ml of *tert.*-butylmethyl ether. After stirring for 5 min and centrifuging at 2200 *g* for 10 min, the organic phase was separated. The extraction was repeated combining the

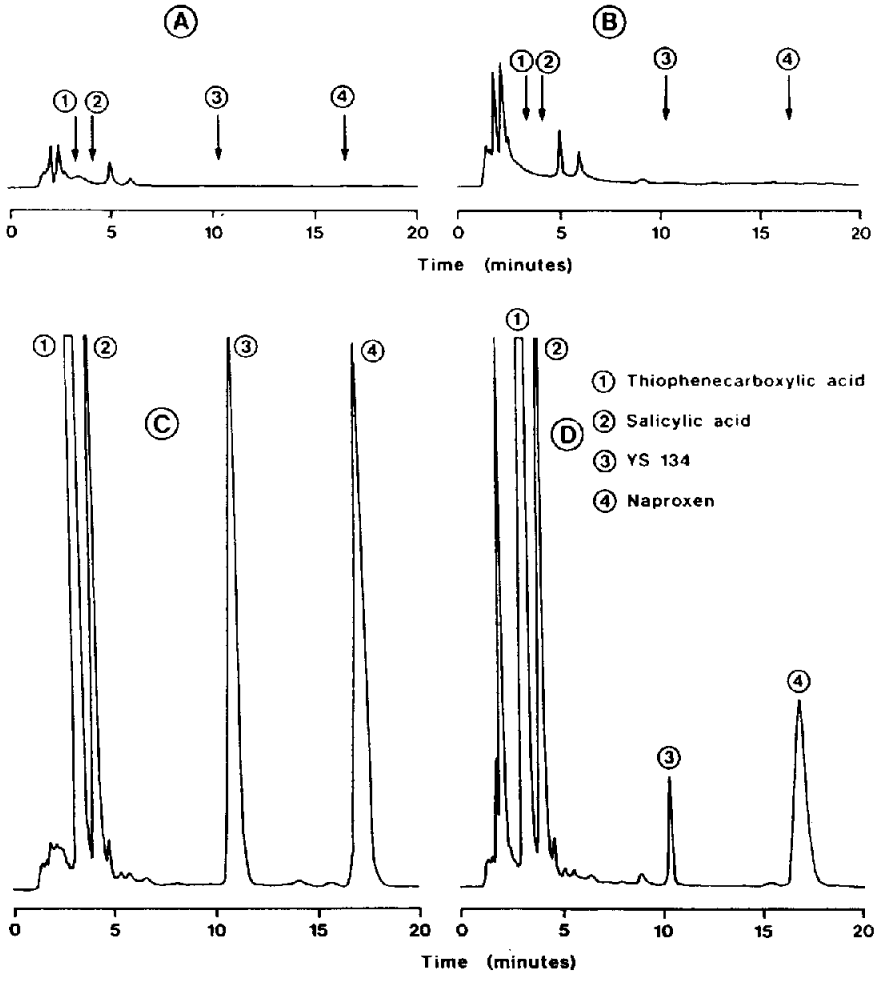



Fig. 2. HPLC of TBA, salicylic acid (SA), thiophenecarboxylic (TA) and naproxen. (A) Blank plasma; (B) blank urine; (C) a plasma blank to which an authentic standard of each analyte was added (5 $\mu$g/ml); (D) plasma sample of a rat orally treated with TBA (YS 134) (300 mg/kg): TA (6.8 $\mu$g/ml), SA (7.8 $\mu$g/ml) and TBA (0.80 $\mu$g/ml) were detected. Internal standard: 2 $\mu$g/ml.

organic phases, which were evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was redissolved with 0.5 ml of eluent and an aliquot of 20 μl was injected into the analytical column.

The eluent consisted of methanol–0.01 *M* potassium dihydrogenphosphate solution (30:70, v/v) adjusted to pH 3.1 with acetic acid; the flow-rate was 0.5 ml/min; absorbance was monitored at 254 nm; retention time was 3.3 min for TA, 4.1 min for SA, 10.2 min for TBA and 16.3 min for naproxen (Fig. 2).

*Application to rats*

Both sexes of albino Sprague–Dawley rats (175–200 g body weight) were used for the experiment. Before the trial the animals were caged for seven days at 21–22°C and 55–75% relative humidity with a cycle of 12 h light–12 h dark. The animals were orally treated with TBA (300 mg/kg) or ASA (220 mg/kg) by a gastric gavage, both substances being suspended in 2% arabic gum. Six animals per group (three males and three females) were killed at times 0 (baseline), 0.5, 1, 2, 4, 8, 16 and 24 h. Heparinized blood was sampled and centrifuged to obtain plasma. Liver, kidneys, lungs, myocardium, gastric wall and intestinal wall were also sampled from the animals killed 0, 1, 2, 4, 8 and 16 h after dosing.

All the samples were stored in a freezer at −20°C and assayed as described above.

TABLE I

EXTRACTION RECOVERY, LINEARITY AND REPRODUCIBILITY OF THE ASSAY IN BLANK PLASMA OF RATS FORTIFIED WITH TBA, SA AND TA

Mean values of three findings.

| Ammount added (μ/ml) | Amount recovered | | C.V. (%) |
|---|---|---|---|
| | μg/ml | % | |
| *TBA* | | | |
| 1 | 0.89 | 88.70 | 4.49 |
| 5 | 4.87 | 97.40 | 4.52 |
| 10 | 9.95 | 99.50 | 1.00 |
| 50 | 48.68 | 97.40 | 5.86 |
| Mean | | 95.75 | 5.01 |
| *SA* | | | |
| 5 | 4.81 | 96.20 | 3.12 |
| 10 | 9.87 | 98.70 | 1.93 |
| 20 | 19.95 | 99.80 | 1.30 |
| 50 | 48.83 | 97.70 | 2.01 |
| 100 | 100.59 | 100.60 | 2.31 |
| 150 | 148.87 | 99.25 | 2.04 |
| Mean | | 98.71 | 1.59 |
| *TA* | | | |
| 5 | 4.58 | 91.60 | 1.97 |
| 10 | 9.17 | 91.70 | 2.62 |
| 20 | 18.29 | 91.40 | 4.10 |
| 50 | 44.99 | 89.97 | 1.56 |
| 100 | 91.37 | 91.37 | 1.70 |
| 150 | 135.94 | 90.62 | 3.03 |
| Mean | | 91.11 | 0.74 |

## RESULTS AND DISCUSSION

Linearity was ascertained in the ranges 1–50 μg/ml TBA and 5–150 μg/ml SA and TA (Table I).

Extraction recovery measured in the above ranges was on average 95.75% for TBA, 98.71% for SA and 91.11% for TA (Table I).

In the whole analysis of TBA extracted from plasma, the inter-assay coefficient of variation (C.V.) ranged from 1.00 to 5.86% and the intra-assay C.V. was 5.01%. In the case of SA and TA the intra-assay C.V. ranged from 1.30 to 3.12 and from 1.56 to 4.10%, respectively, and the intra-assay C.V. was 1.59 and 0.74%, respectively.

The lowest detectable plasma concentration associated with a C.V. less than 10% proved to be 0.2 μg/ml for TBA and 0.5 μg/ml for SA and TA.

Analysis can be carried out with a good selectivity, as analytical peaks are well separated and no interfering endogenous peaks are detected (Fig. 2).

Fig. 3 and Table II show the main data regarding plasma and tissue concentration after oral administration of TBA and ASA. The SA concentration profile in plasma and tissues after oral administration of TBA proved to be delayed, mainly in the absorption phase, compared with ASA. The absorption and the reversibility of TBA are in fact two processes preceding and conditioning the kinetics of plasma SA concentra-

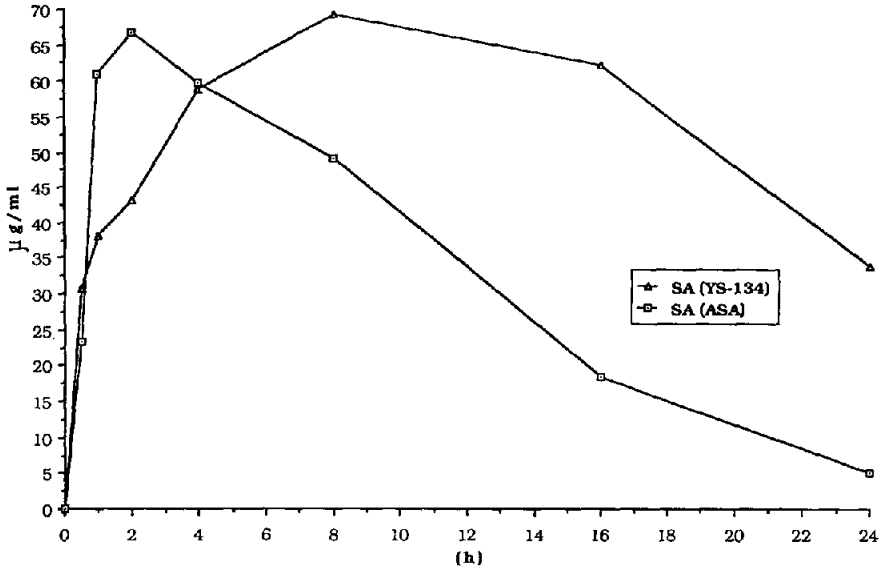



Fig. 3. Plasma concentration of salicylic acid (SA) in rats after oral administration of TBA (300 mg/ml) and acetylsalicylic acid (ASA) (220 mg/ml). Mean values of six findings.

TABLE II

TISSUE CONCENTRATION OF SA AFTER ORAL ADMINISTRATION TBA (300 μg/kg) IN RATS

N.E. = not evaluatable in that concentration (below the detection limit of the method).

| Time (h) | Concentration (mean ± S.D., $n$ = 6) (μg/g of wet tissue) | | | | | |
|---|---|---|---|---|---|---|
| | Kidneys | Liver | Myocardium | Lungs | Gastric wall | Intestinal wall |
| 1 | 4.9 ± 1.7 | 9.2 ± 1.4 | 18.3 ± 6.7 | 8.0 ± 3.1 | 432.8 ± 356.4 | 379.3 ± 107.6 |
| 2 | 6.0 ± 1.1 | 11.8 ± 1.3 | 34.4 ± 6.0 | 10.5 ± 4.0 | 268.1 ± 164.0 | 140.7 ± 48.8 |
| 4 | 7.4 ± 2.1 | 15.1 ± 2.8 | 39.4 ± 6.1 | 16.8 ± 1.7 | 73.8 ± 87.4 | 12.7 ± 11.7 |
| 8 | 9.4 ± 1.2 | 17.0 ± 1.5 | 38.5 ± 5.0 | 18.5 ± 2.0 | 18.4 ± 28.9 | N.E. |
| 16 | 3.3 ± 1.7 | 15.1 ± 3.2 | 29 ± 5.7 | 7.5 ± 3.0 | N.E. | N.E. |

tion. The administration of TBA allows a higher bioavailability of SA to be achieved than after dosing with ASA.

The analytical procedures described in this study allow the assay of TBA, SA and TA to be carried out in plasma and tissues of rat using a simple isocratic analysis. The wavelength of 254 nm was selected in order to achieve the most intensive detection of parent drug, the component which disappears quickest.

This method is easy to apply, requires solvents of low cost and uses appparatus which is very common in pharmacokinetic laboratories. A skilful operator can process more than 60 samples a day using an automatic sample injector. This method was applied in a comprehensive pharmacokinetic investigation in the rat and in a bioavailability study in human which is now in progress.

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