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DETERMINATION OF OXAZOLIDINE-2-THIONES IN BIOLOGICAL FLUIDS IN THE ppb* RANGE

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SUMMARY

A simple, cheap and rapid method for the quantitation of oxazolidine-2-thiones in biological fluids and fodder in the concentration range 1–1000 ppb is described. Oxazolidine-2-thiones are extracted from 2 ml fluid using cyclohexane saturated with phenylmercuric acetate, derivatized with pentafluorobenzoyl chloride and analysed by fused-silica open tubular capillary gas chromatography with electron-capture detection.

INTRODUCTION

Different countries of the E.E.C. prohibit the use of thyreostatic drugs (also called anti-hormones, AH) in cattle breeding. The drugs most frequently used are of the thiouracil type, in particular, 5-methyl-2-thiouracil. A method for the determination of these drugs in biological material was worked out[1] and accepted by the E.E.C.[2].

Next to the synthetic thyreostatic drugs, a group of natural sulphur compounds are of most interest. These are hydrolysis products of glucosinolates present in plants of the Cruciferae and related families[3,4]. The hydrolysis is catalysed generally by the enzyme myrosinase (thioglucoside glucohydrolase E.C. 3.2.3.1). Non-enzymatic hydrolysis[5] and hydrolysis by bacterial enzymes of the gastro-intestinal tract have also been described[6,7]. The mechanism of hydrolysis is complex. Several compounds can be formed depending on the structure of the glucosinolate (more than 65 glucosinolates are known) and the hydrolysis conditions. Most of these compounds may act as goitrogens by metabolisation to thiocyanate ion[8]. The determination of thiocyanate in biological material was described previously[9].

Oxazolidinethiones, Fig. 1, a class of cyclic compounds, are formed when an appropriately located hydroxyl substituent is present in the glucosinolate. Also called goitrins, these compounds are potent goitrogens, which are not counteracted by iodine as is thiocyanate. The best known member of this group, 5-vinyloxazolidine-2-thione (goitrin, VTO), has 133% of the goitrogenic activity of propylthiouracil in

* Throughout this article, the American billion (10^9) is meant.

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man[4]. The amount of progoitrin (the glucosinolate from which goitrin is liberated) in most parts of plants is small. In contrast, some Cruciferae seed meals contain progoitrin equivalent to 1 % goitrin[10]. Rape-seed meal used as a protein supplement in animal feeds has produced some adverse effects, due to the presence of goitrogens.

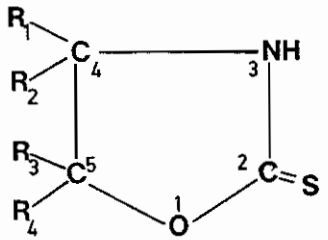

Fig. 1. Structural formula of oxazolidine-2-thiones. 5-VTO: R_1, R_2, R_3 = H; R_4 = vinyl. 5-MTO: R_1, R_2, R_3 = H; R_4 = methyl. TO: R_1, R_2, R_3, R_4 = H. 4-IPTO: R_1 = isopropyl; R_2, R_3, R_4 = H.

When progoitrin is ingested by a lactating cow, VTO appears in the milk[11]. The feeding of milk with even low concentrations of VTO (50–100 ppb) to rats over long periods causes enlarged thyroids[12], although the goitrogenic activity of VTO in the rat is only 2 % of that in man[4]. Moreover, the iodine content of milk is decreased due to the influence of goitrogens[13,14]. Goitrogenous milk may be harmful to infants and children consuming milk in large quantities.

In poultry the synthesis of thyroid hormones is depressed by VTO[15–17]. Growth retardation of chickens fed rape-seed meal was also observed[18]. The appearance of a "fishy" taint to the eggs of certain breeds was correlated with the presence of thyreostatic drugs in fodder[19–21]. VTO was also found in quail eggs after administration of progoitrin to the animal[22].

The determination of the high levels of glucosinolates[23,24] or their breakdown products[25–28] in plant material has been studied thoroughly and adequate methods were described. Recently, attempts were made to determine the small quantities of VTO in biological material of animal origin by modern chromatographic methods[22,29–32]. Although some authors achieved low detection limits (2–10 ppb) the amount of sample needed is high (50 g or more), the analysis time is long and large amounts of organic solvents are wasted.

In this paper a simple, fast and cheap method is described which permits the detection of several oxazolidine-2-thiones in plasma, urine, milk and fodder in the range 1–1000 ppb. The method is based on the selective extraction of oxazolidine-2-thiones with an organic mercury compound[33] and on the quantitative formation of stable and highly sensitive derivatives with pentafluorobenzoyl chloride (PFB-Cl).

EXPERIMENTAL

Reagents and reference compounds

Phenylmercuric acetate (PMA), PFB-Cl and 2-amino-4,6-pyrimidinedithiol were obtained from Aldrich-Europe (Beerse, Belgium). Cyclohexane was from Fluka (Buchs, Switzerland) and toluene from E. Merck (Darmstadt, G.F.R.). L-5-vinyloxazolidine-2-thione (5-VTO) was purchased from The National Physical Lab. (Teddington, Great Britain).

5-Methyl- (5-MTO), 4-isopropyl- (4-IPTO) oxazolidine-2-thione and oxazo-

lidine-2-thione were synthesised according to Ettlinger[34] by cyclization of the appropriate aminoalcohol with carbon disulphide in dioxane. The products were purified by crystallization from benzene.

All other reagents used were reagent-grade products from different manufacturers.

Solutions

Cyclohexane was saturated with PMA at 60°C. After cooling to room temperature the solution was filtered and the filtrate (PMA solution) stored at room temperature in the dark.

A stock solution of 5% (w/v) PFB-Cl in toluene was prepared by adding 30 μl (48 mg) PFB-Cl to 1 ml toluene. The solution was stored at 4°C. A working solution of PFB-Cl was prepared daily by diluting 100 μl stock solution in 900 μl toluene.

A myrosinase solution was prepared according to Wrede[35]. A 10-g amount of ground mustard seed was homogenized with 60 ml water, stirred for 30 min and centrifuged at 10.000 rpm (12,000 *g*). The supernatant was mixed with an equal volume of 90% ethanol. The mixture was centrifuged, the precipitate washed with 20 ml of 70% ethanol and centrifuged again. The ethanol was removed and the remainder taken up in 10 ml water. This solution contains *ca.* 20 mg enzyme per ml solution.

Other reagents used were potassium hydroxide solution (5%, w/v) and phenolphthalein solution (0.1 g% in ethanol).

Standard solutions

A combined stock standard solution of 5-MTO, TO and 5-VTO was prepared by dissolving 4 mg of each component in 50 ml toluene. The addition of 25 μl of this solution to 2 g of a biological matrix is equivalent to a concentration of 1 ppm. From this stock solution, standard solutions equivalent to 250, 500 and 750 ppb were prepared by dilution in toluene. Ten-, hundred- and thousand-fold dilutions of this series were also prepared in toluene.

A stock internal standard solution of 4-IPTO was prepared by dissolving 7 mg of 4-IPTO in 50 ml toluene. The response of the electron-capture detector (ECD) to this solution is of the same magnitude as that to a standard solution equivalent to 1 ppm.

Ten-, hundred- and thousand-fold dilutions of the internal standard solution were prepared in toluene.

Chromatographic conditions

The gas chromatograph used was a Varian 3700, equipped with a capillary injection system and an ECD. Integration was performed with a Varian Vista 401 chromatographic data system. Chromatograms were plotted with baselines from raw data stored on floppy disc. The electrometer settings on the 3700 were: range 10; attenuation 8. Attenuation of the chromatograms was performed with the integrator. The column was a fused-silica capillary (50 m × 0.23 mm I.D.) coated with CP Sil 5 (OV-1, SE-30 analogue) from Chrompack (Middelburg, The Netherlands).

Hydrogen was used as carrier gas at a rate of 1 ml/min. The splitting ratio was 1:25. Nitrogen was used as make-up gas for the ECD at 30 ml/min.

The column, injector and detector temperatures were 170°C, 220°C and 310°C respectively. The retention times of PFB derivatives of oxazolidine-2-thiones under these conditions were: 5-MTO, 7.5 min; TO, 7.7 min; 5-VTO, 8.9 min and 4-IPTO, 10.2 min.

Apparatus

The following apparatus was used: centrifuge, water-bath, homogenizer (Ultra-turrax), Reacti-term heating module (Pierce), round-bottomed extraction tubes (15 ml, *ca.* 10 cm × *ca.* 15 mm I.D.; 8 ml, *ca.* 9 cm × *ca.* 10 mm I.D.) and conical reaction tubes (5 ml, *ca.* 7 cm × *ca.* 10 mm I.D.).

During the course of this work a handy and inexpensive nitrogen jet evaporator was constructed (see Fig. 2). The lamp hood was removed from a commercial desk-lamp with movable arm. It was replaced by a biconical connection tube (Moplen, 878 Kartell). One end of the tube was connected to a nitrogen cylinder. Over the other end a piece of silicone tubing (*ca.* 1 cm I.D.) closed by a thin rubber stop (*ca.* 1 cm) was slipped. Jets were constructed from Venoject multi-sample needles [Terumo; 20 gauge × 15 in. thin wall (0.9 × 40 mm)], silicone tubing (*ca.* 2 mm O.D., 0.5 mm I.D.) and PTFE tubing (1.5 mm O.D., 0.5 mm I.D.). The desired amount of jet was inserted through the rubber stop. The movable arm allows satisfactory adjustment of the nitrogen jets during evaporation. It is handy to use this system in combination with a Reacti-term heating module.

Analytical procedure

A 2-g sample (*e.g.*, plasma, urine, serum, milk or any aqueous extract) was placed into a 15-ml extraction tube with screw stopper. One or two drops of phenolphthalein solution were added and the solution made alkaline by adding small amounts of 5% potassium hydroxide solution. PMA solution (5 ml) was added and the tube shaken vigorously for 1 min. Phases were separated by centrifugation at 2000 rpm (500 *g*) for 10 min. The clear upper phase was transferred into a 8-ml silanized extraction tube. The volume was reduced to *ca.* 1 ml under a jet of nitrogen. Depending on the concentration range investigated, an appropriate internal standard solution (25 µl) was added. For the analysis of concentrations less than 100 ppb the contents of the tube were transferred to conical 5-ml reaction tubes. The solvent was evaporated to dryness under a jet of nitrogen. The derivatization parameters for the three different concentration ranges are summarized in Table I. The residue was taken up in an appropriate amount of toluene, PFB-Cl added and the tube heated at 100°C for 1 h in a Reacti-term heating module. After cooling, 1 µl of the contents was injected into the gas chromatograph.

Pre-treatment of fodder

A 0.5-g amount of fodder was homogenized with 19.5 ml buffer pH = 6 and heated at 100°C for 3 min. Myrosinase solution (0.5 ml) was added and the mixture incubated at 40°C for 24 h. The mixture was centrifuged at 10,000 rpm (12,000 *g*) for 10 min. A 100-µl volume of the supernatant was mixed with 1.9 ml water. The aqueous solution (equivalent to 2.5 mg fodder) was extracted with PMA solution as described above.

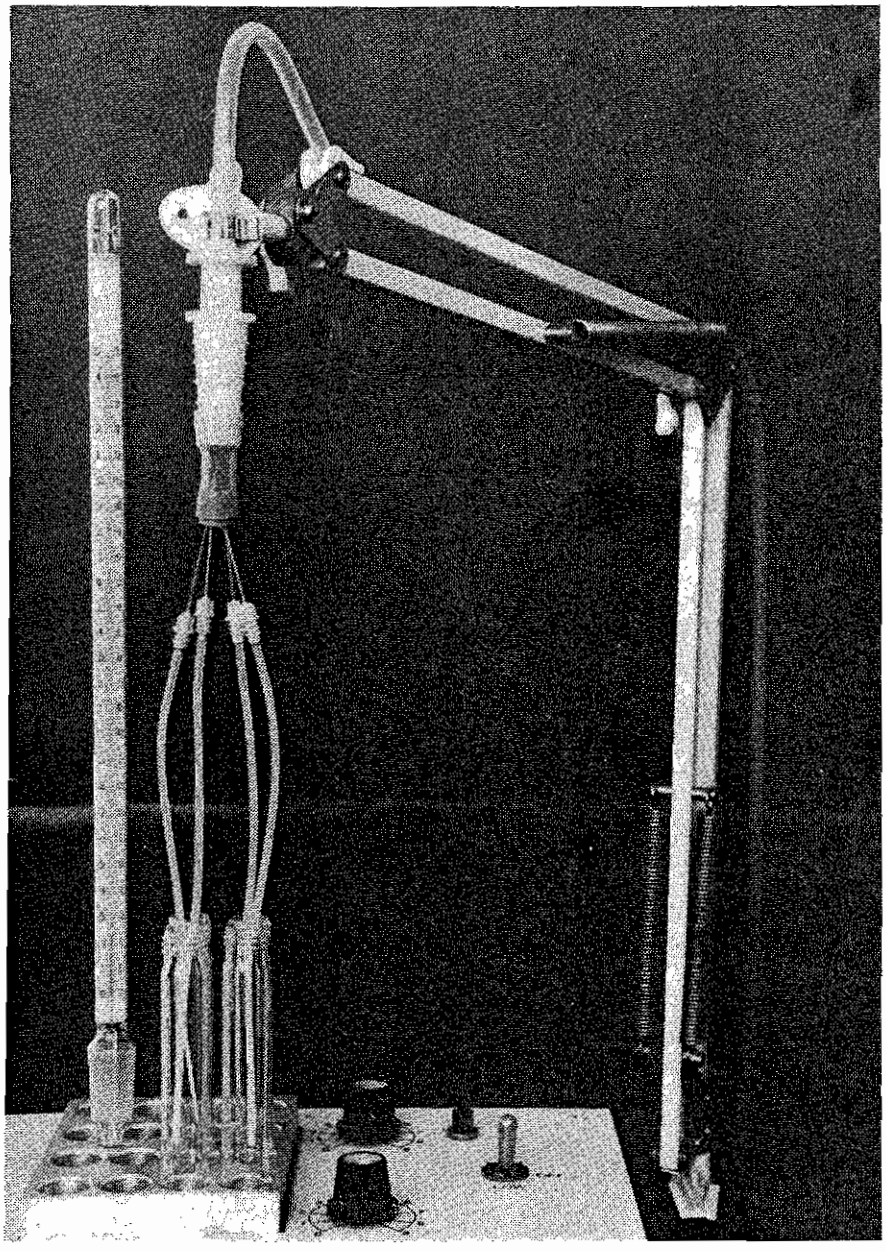

Fig. 2. Nitrogen jet evaporator with movable arm.

TABLE I

DERIVATIZATION PARAMETERS FOR THE THREE CONCENTRATION RANGES

Concentration range (ppb)	*Derivatization volume (μl)*	*PFB-Cl added (μl)*	*Medium*
100–1000	1000	10 (stock)	Fodder, urine
10–100	100	10 (1/10)	Plasma, urine
1–10	50	2 (1/10)	Milk, plasma

RESULTS AND DISCUSSION

Pentafluorobenzoylation conditions

Pentafluorobenzoylation is usually carried out with a basic catalyst such as

trimethylamine, pyridine or potassium carbonate[36]. The use of these basic catalysts in the derivatization of oxazolidine-2-thiones was not successful. Better results were obtained using extractive benzoylation: the derivatization is carried out by adding PFB-Cl to cyclohexane, saturated with triethanolamine. The cyclohexane phase is then shaken with an aqueous basic solution. The upper (organic) phase is transferred into another vial, dried and evaporated to dryness. Unfortunately, severe losses of oxazolidine-PFB derivatives occur during evaporation. The extractive benzoylation procedure was used successfully by Delbeke and Debackere[37] in the determination of butinilicaine in horse plasma and urine.

Since the excess of PFB-Cl could not be removed by evaporation without losses of the derivatives, we tried to keep the concentration of PFB-Cl small so that it would not interfere on the chromatogram. It was found that oxazolidine-2-thiones can be derivatized with PFB-Cl by heating the products in solvents such as cyclohexane and toluene. Small amounts (*ca.* 1%) of more polar solvents such as methanol or ethyl acetate disturb the reaction.

The reaction rate was studied as a function of temperature, solvent and concentration of PFB-Cl using standard solutions equivalent to 10, 100 and 1000 ppb. During this study an appropriate amount of lindane was added to the mixture as an additional internal standard (retention time = *ca.* 9.4 min) because of its invariable peak area. The ratios of the peak areas of oxazolidine-2-thiones to lindane and of 5-MTO, TO and 5-VTO to the internal standard 4-IPTO were calculated in all experiments.

In Figs. 3 and 4 derivatization yield *versus* time curves for 5-VTO at different temperatures and different concentrations of PFB-Cl are given. Derivatization is complete after heating for 1 h at 100°C and using a concentration of PFB-Cl of 480 μg/ml. All four oxazolidine-2-thiones studied gave similar derivatization yield curves. Therefore the peak area ratio of 5-MTO, TO and 5-VTO to the internal standard is stable after 20 min (derivatization yield *ca.* 80%). The concentration of PFB-Cl used did not interfere with the chromatograms. No significant differences were found

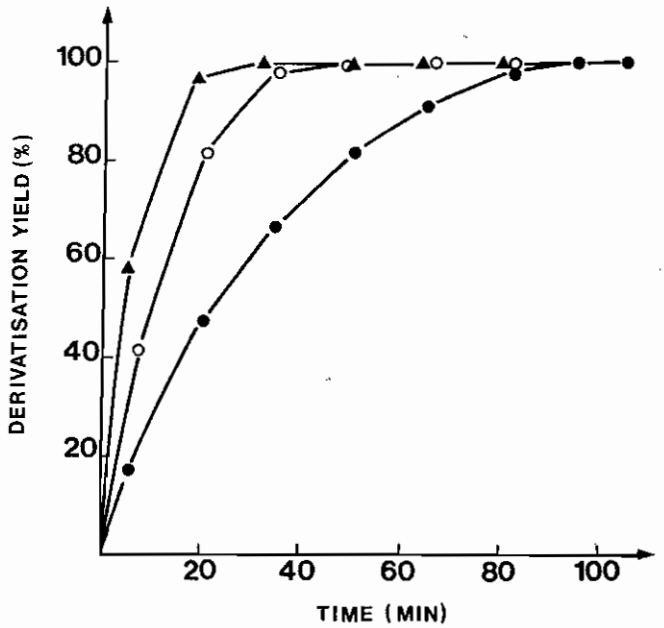

Fig. 3. Derivatization yield of 5-VTO at 100°C as a function of time and increasing amounts of PFB-Cl (●, 120 μg/ml; ○, 480 μg/ml; ▲, 2400 μg/ml).

between derivatizations in toluene or cyclohexane. For derivatizations in small amounts of solvent ($\leqslant 100$ μl) toluene is preferred due to its higher boiling point. Once formed, the solutions of the derivatives are stable at room temperature for weeks.

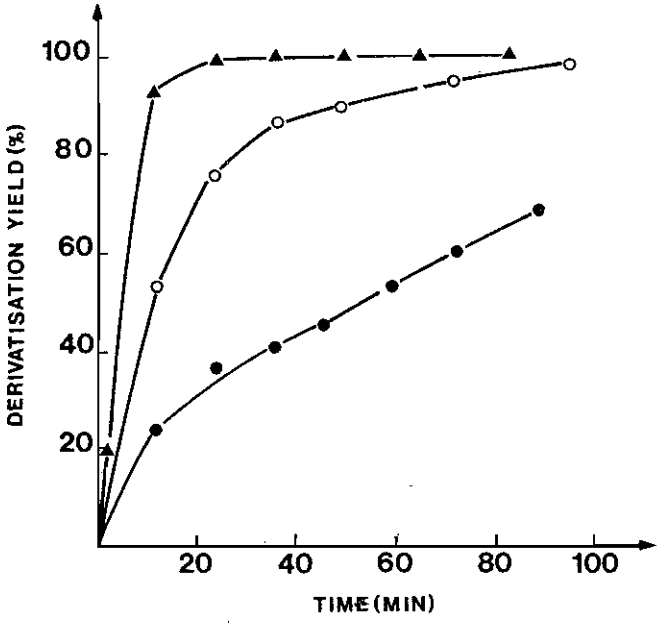

Fig. 4. Derivatization yield of 5-VTO at a concentration of 2400 μg PFB-Cl per ml as a function of time at different temperatures (●, 55°C; ○, 75°C; ▲, 100°C).

Calibration graphs

For the accurate determination of oxazolidine-2-thiones in the concentration range 1–1000 ppb three series of four standard solutions and three internal standard concentrations were used. The ranges covered are: 1–10 ppb; 10–100 ppb and 100–1000 ppb. Higher concentrations were determined by appropriate dilution of the sample. The response factors of the three internal standard concentrations relative to the three ten-fold dilutions of four standard solutions were determined by carrying out the derivatization as described in Table I.

In each series linear calibration curves were obtained by plotting the ratio of the peak area of the oxazolidine-2-thiones to the peak area of the internal standard against the amount of oxazolidine-2-thiones. A calibration graph for the series 1–10 ppb is given in Fig. 5. Since all standard and internal standard solutions are prepared by similar dilution from the two stock solutions the three calibration curves should be identical. In Fig. 6 the logarithm of the ratio of the peak area of 5-VTO (multiplied by 100 for 100–1000 ppb and by 10 for 10–100 ppb) to the internal standard is plotted against the logarithm of the concentration of 5-VTO. The curves illustrate the linearity of the method over the concentration range studied (1–1000 ppb).

Extraction and clean-up

In all methods hitherto described, 5-VTO was extracted from biological material with solvents such as diethyl ether, ethyl acetate and dichloromethane[29–31,38] (see Table IV). The amount of sample used (*e.g.*, 300 ml milk) required en expensive amount of solvent (*e.g.*, 2 × 500 ml solvent per sample). Special care to avoid

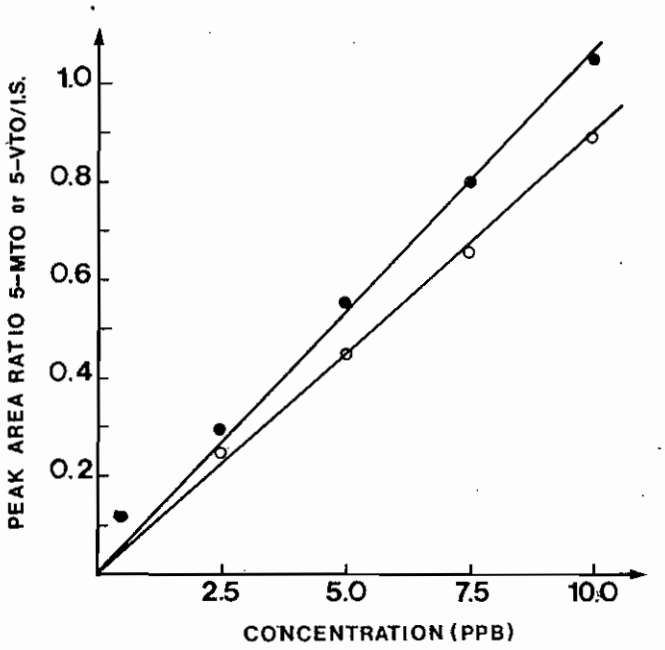

Fig. 5. Calibration graph of 5-methyloxazolidine-2-thione (○) and 5-vinyloxazolidine-2-thione (●) using 4-isopropyloxazolidine-2-thione as internal standard in the concentration range 1–10 ppb (40–400 pg/ml).

emulsions had to be taken during successive extractions. The concentration of high quantities of solvent to small volumes for injections in gas chromatography (GC) or liquid chromatography (LC) was very time-consuming and raised problems of interference from solvent contaminants. Since extractions are not selective, a clean-up procedure was needed, high-performance liquid chromatography (HPLC), thin-layer

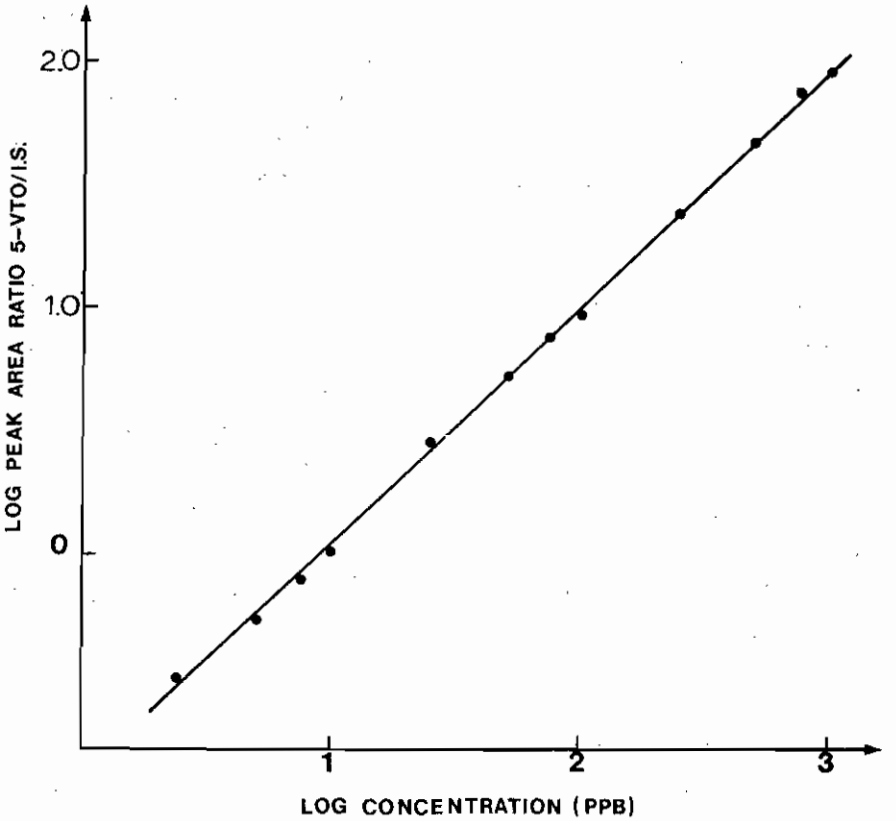

Fig. 6. log–log calibration graph of 5-vinyloxazolidine-2-thione using three internal standard dilutions, showing linearity in the concentration range 1–1000 ppb (correlation: $y = 0.96x - 0.924$; $r = 0.9998$).

chromatography (TLC) or column chromatography, before detection was performed.

In this investigation extraction and clean-up are carried out in a single step. Therefore we searched for a highly selective extraction procedure. Thyreostatic drugs were found to form very specific complexes with mercury ions[33]. Several organic mercury compounds, preferably soluble in organic solvents such as toluene or cyclohexane, were tested. It was found that a solution of phenylmercuric acetate in cyclohexane (PMA solution) could extract oxazolidine-2-thiones from alkaline aqueous solution with an excellent yield.

In order to compare the extraction yield using PMA solution with those obtained using organic solvents, the distribution coefficients of three oxazolidine-2-thiones between aqueous solutions at three different pH values and a number of selected organic solvents were determined. A 25-μl volume of a standard solution (equivalent to 750 ppb or 1.5 μg 5-VTO, 5-MTO and TO) was placed into 15-ml extraction tubes. The solvent was evaporated under a jet of nitrogen and the residue was dissolved in 5 ml aqueous solution (0.1 *N* HCl, pH 1; 0.1 *M* phosphate buffer, pH 7 or 0.1 *N* NaOH, pH 13). The solution was extracted twice with 5 ml solvent. Both extracts were evaporated to dryness and redissolved in 1 ml cyclohexane. Internal standard was added and derivatization was carried out as described before. The results are given in Table II.

With hexane and cyclohexane the extraction yields are very low (<2%). With diethyl ether approximately 40% VTO is extracted at neutral pH. The extraction yields of the other oxazolidinethiones are lower. In an acidic medium, chloroform gives the best results, 71%, 58% and 15% VTO, MTO and TO respectively being extracted in a single equilibration. In a neutral medium good results were obtained with ethyl acetate (75% VTO, 64% MTO and 40% TO), even better with PMA solution. In an alkaline medium oxazolidinethiones are ionized and practically no extraction is obtained. Due to the complexation with mercury, 91% MTO, 87% TO and 92% VTO are transferred into the organic phase in a single equilibration of equal volumes of upper and lower phase.

TABLE II

DISTRIBUTION COEFFICIENTS OF OXAZOLIDINE-2-THIONES BETWEEN AQUEOUS SOLUTION AND ORGANIC SOLVENTS AT DIFFERENT pH VALUES

Solvent	*D at pH = 1*			*D at pH = 7*			*D at pH = 13*		
	*5-MTO**	*TO*	*5-VTO*	*5-MTO*	*TO*	*5-VTO*	*5-MTO*	*TO*	*5-VTO*
Diethyl ether	0.050	0.029	0.081	0.190	0.056	0.61	0.092	0.005	0.013
Cyclohexane	0.004	0.007	0.004	0.005	0.005	0.017	0.003	0.002	0.006
Hexane	0.002	0.006	0.006	0.002	0.002	0.012	0.002	0.001	0.004
Benzene	0.100	0.023	0.327	0.014	0.040	0.473	0.002	0.001	0.003
Ethyl acetate	0.472	0.187	0.758	1.83	0.65	3.54	0.003	0.012	0.028
Chloroform	1.400	0.17	2.440	1.44	0.27	3.00	0.003	0.003	0.004
PMA solution**	0.016	0.016	0.019	3.66	1.64	9.68	10.05	6.63	12.15

* Concentration of oxazolidine-2-thione used = 750 ppb.

** Cyclohexane saturated with phenylmercuric acetate.

Removal of excess of phenylmercuric acetate

The injection of 1 μl PMA solution into the gas chromatograph produces a tailing peak with a retention time of *ca.* 6 min. Whether this peak can be attributed to phenylmercuric acetate (PMA) itself or to the breakdown products[39] was not investigated. Since the PMA solution is concentrated from 5 ml to 1000 μl or 50 μl the intense peak of PMA, eluting just before the peaks of interest, will disturb the analysis. Fortunately, most of the PMA is removed spontaneously during the extraction. In the range 10–1000 ppb no or very little interference from PMA is observed. At lower concentrations, PMA can be removed after derivatization by adding a few grains of 2-amino-4,6-pyrimidinedithiol to the vial and heating for 10 min at 100°C.

Recovery of oxazolidine-2-thiones

The recovery of three oxazolidine-2-thiones, *i.e.*, 5-MTO, TO and 5-VTO, was determined in milk, plasma and urine at three different concentrations: 750, 75 and 7.5 ppb. Standard solutions (25 μl) were added to 15-ml extraction tubes, the solvent was evaporated and the residue taken up in 2 ml milk, plasma, urine or distilled water. The procedure was carried out as described above.

From the eighteen extractions from distilled water (six times for each of three concentrations) a mean extraction yield ± standard deviation of 86 ± 3.5% was calculated for all three oxazolidine-2-thiones. This agrees with the theoretical extraction yield calculated from the distribution coefficients (Table II) and the amount of PMA solution recovered from the upper phase (4.5 ml of 5 ml or *ca.* 90%).

The recoveries of oxazolidine-2-thiones from urine, plasma and milk were corrected for a mean extraction yield of 86% (Table III). In urine the three oxazolidine-2-thiones gave the highest recovery at the 750 ppb level. On the contrary, in milk, recoveries at concentrations of 7.5–75 ppb were higher than those at 750 ppb. In plasma the recoveries of 5-MTO and 5-VTO were similar and constant over the concentration range studied; however, the recovery of TO was significantly lower than that observed for the other drugs.

Detection limit

Although the physiological activity of oxazolidine-2-thiones in animal and

TABLE III

RECOVERY OF OXAZOLIDINE-2-THIONES FROM BIOLOGICAL FLUIDS

Six experiments were carried out in each case.

Concentration added (ppb)	*Fluid*	*Recovery (% ± standard deviation)*		
		5-MTO	*TO*	*5-VTO*
750	Urine	90 ± 2	89 ± 5	93 ± 2
750	Plasma	84 ± 4	78 ± 7	84 ± 4
750	Milk	79 ± 6	84 ± 8	82 ± 6
75	Urine	76 ± 5	68 ± 7	84 ± 5
75	Plasma	80 ± 8	68 ± 11	86 ± 4
75	Milk	94 ± 2	93 ± 3	92 ± 6
7.5	Urine	77 ± 12	81 ± 12	82 ± 12
7.5	Plasma	89 ± 10	71 ± 20	85 ± 13
7.5	Milk	100 ± 7	96 ± 10	90 ± 10

man has not been fully investigated, it can be assumed that the effect of blood concentrations less than 1 ppb on thyroid function will be of minor importance. Therefore, the determination of goitrins at the sub-ppb level has not been studied in detail. In preliminary experiments it was demonstrated that the detection limit of this method without special purification of the reagents is smaller than 1 ppb. Standard solutions equivalent to 0.75 ppb 5-MTO, TO and 5-VTO were added to plasma and urine. The chromatograms were compared with those obtained for the same plasma and urine samples without addition. In Fig. 7 all three oxazolidine-2-thiones are detected in urine at the level of 0.75 ppb. For plasma an analogous result was found.

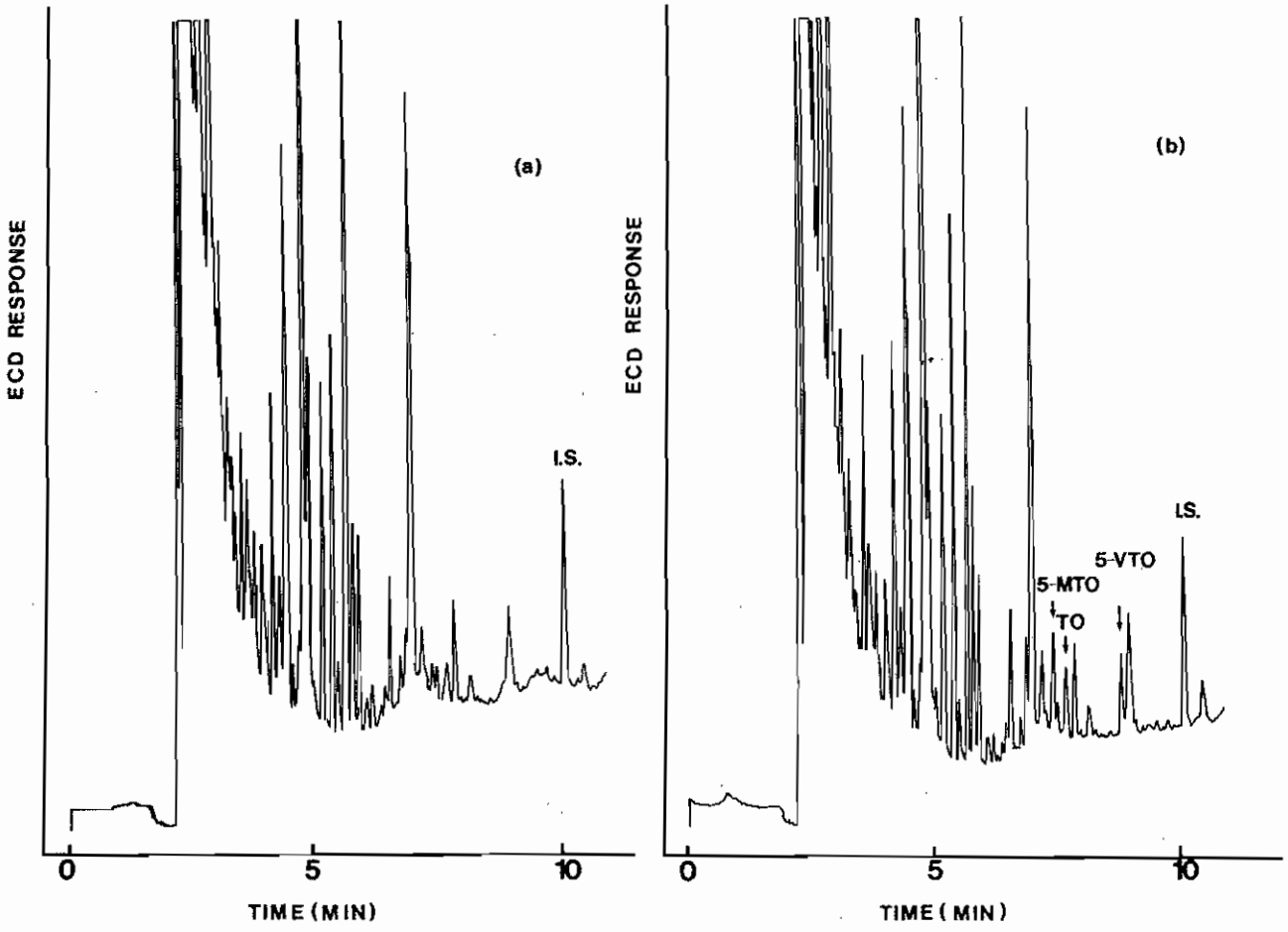

Fig. 7. Gas chromatogram of (a) a urine sample and (b) the same urine sample spiked with 0.75 ppb 5-MTO, TO and 5-VTO. I.S. = Internal standard.

The minimum detectable amount of 5-VTO, defined as that which gives a response equal to twice the noise, was calculated to be 0.2 ppb (0.4 ng in 2 g sample).

Elimination of 5-MTO after intraruminal administration to a goat

After morning milking, 400 mg of 5-MTO, dissolved in 250 ml of water, were infused intraruminally by way of a stomach tube to a lactating goat (50 kg body weight, yielding 1 l milk per day). The goat had a chronically implanted polyethylene catheter in a jugular vein and before the drug was administered the urine bladder was catheterized with a Foley catheter. Urine was collected continuously. Heparinized blood, urine and milk sample were taken at 2 h, 4 h, 6 h, 8 h and 9 h post-infusion.

All samples were analysed as described above and corrected for a mean extrac-

tion yield of 86 %. The results are given as a plot of the logarithm of the concentration of 5-MTO (ppm) *versus* time in Fig. 8. Parallel straight lines were obtained for urine. plasma and milk. From these curves the average half-life of 5-MTO in goat after single intraruminal administration was calculated as 75 min.

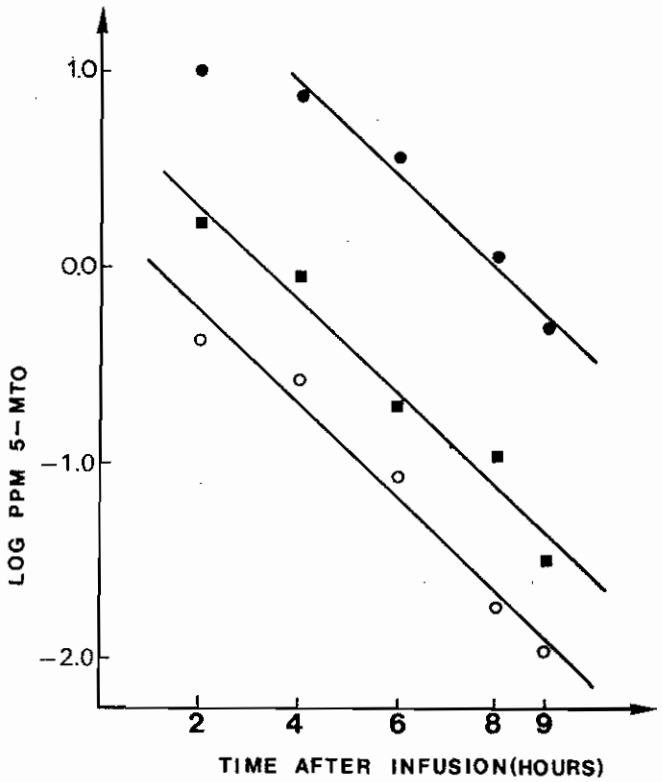

Fig. 8. Graph showing the elimination of 5-MTO in milk (○), plasma (■) and urine (●) following intraruminal administration of 400 mg 5-MTO. The 5-MTO amounts of urine were corrected to a creatinine concentration of 50 mg%.

During the experiment (0–9 h post-infusion), 3.4 g of 5-MTO (0.85 % of the administered dose) were excreted via the urine. In milk, only 0.13 mg 5-MTO (0.03 % of the dose) were recovered over the experimental period.

In comparison with plasma, the 5-MTO was ten-fold concentrated in urine, while in milk its concentration was diluted four-fold.

Goitrin concentration of some samples

During the course of this work some samples of plasma, urine and milk of different origins were collected and used for testing the analytical procedure. In most samples no or only small amounts of oxazolidine-2-thiones were found. The commercial milk used was whole AA milk*, packed in Tetrabrik and prepared according to a UHT (ultra high temperature) procedure. In this milk various concentrations of 5-VTO between 3 and 8 ppb were found. A chromatogram of a milk sample is given in Fig. 9. Surprisingly high 5-VTO concentrations were found in plasma and urine samples of some non-lactating cows at the veterinary faculty, between 15 and 200 ppb in plasma and between 80 and 250 ppb in urine. The chromatograms of a plasma

* AA milk is milk of high bacteriological quality.

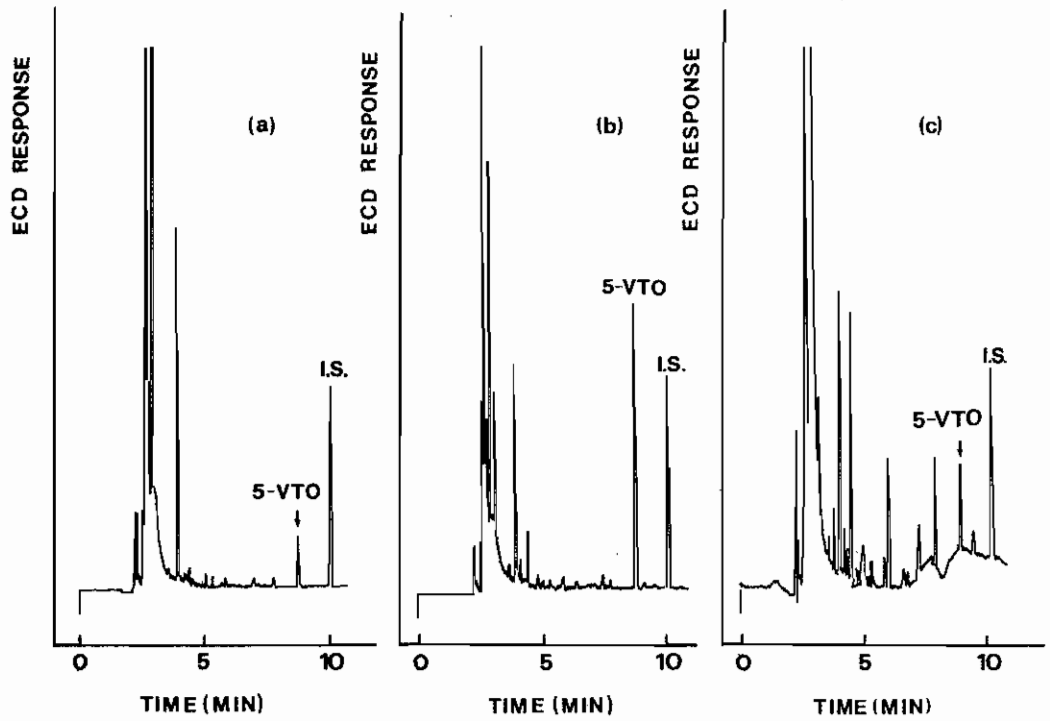

Fig. 9. Gas chromatograms of (a) a plasma sample (30 ppb 5-VTO), (b) a urine sample (160 ppb 5-VTO) and (c) a milk sample (5 ppb 5-VTO).

and urine sample are given in Fig. 9. The fodder of those cows was analysed and progoitrin, equivalent to 1.2 g goitrin per kg fodder, was found. The cows received approximately 5 kg of this commercial fodder per day. According to the manufacturer, *ca.* 5% rape-seed meal was added to the fodder.

Comparison with previously published methods

In Table IV the method described is compared with methods previously de-

TABLE IV

COMPARISON OF THE DESCRIBED METHOD WITH PREVIOUS METHODS

Ref.	*Biological material*	*Method*	*Detection limit (ppb)*	*Amount of sample*	*Amount of solvent*
31	Milk	HPLC(UV)	1	300 ml	Dichloromethane, 1120 ml; hexane, 10 ml
30	Milk	HPLC(UV)	2	50 ml	Diethyl ether, 540 ml; hexane, 12 ml
29	Milk	GLC	2000	50 ml	Diethyl ether, 540 ml; hexane, 12 ml
38	Milk	TLC(UV)	$\geqslant$1000	300 ml	Ethyl acetate, 1000 ml; chloroform, 60 ml
32	Meat	GLC(FID)	1000	100 g	Hexane, 400 ml; ethanol; 500 ml; dichloromethane, 440 ml
22	Eggs	TLC	10	50 g	Diethyl ether, 600 ml; light petroleum, 50 ml
This work	Milk, plasma, urine	Fused-silica open tubular capillary GC (ECD)	< 1	2 g	Cyclohexane, 5 ml

scribed for analysis of 5-VTO in biological material. The comparison is based on the nature of the biological material analysed, the kind of chromatographic method used, the detection limit claimed and the amount of sample and organic solvent used.

The high sensitivity of the ECD for pentafluorobenzoyl derivatives, in combination with the highly selective mercury extraction procedure, allows a very low detection limit while analysing a smaller sample. Moreover, the amount of organic solvent used is at least 100 times less than those used by previous authors. This means that the method is approximately 100 times more economical in solvent and also shortens the analysis time. The number of samples that can be analysed by this method per day is limited by the time required for a chromatographic run (12 min).

The method described here is not compared critically with other methods for analysis of 5-VTO in fodder[25–28] since possible breakdown products of progoitrin are determined simultaneously with 5-VTO. For analyses of oxazolidine-2-thiones in fodder, our method is as fast and as sensitive as any other method hitherto described.

CONCLUSIONS

The fused-silica open tubular capillary GC–ECD method described permits the rapid, simple and accurate determination of oxazolidine-2-thiones in body fluids and fodder of animals at the ppb range. In comparison with existing methods, a lower detection limit (< 1 ppb) is reached for analyses of smaller samples (2 g).

The analysis capacity of this method (30–40 samples per gas chromatograph per day) permits extension of the survey of these thyreostatic drugs to materials other than plant material. So this method may contribute to the study of the fate of these drugs throughout the food chain. Adverse effects produced by these drugs (*e.g.*, growth retardation, egg taint) may be of considerable economical importance.

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REFERENCES

1 H. F. de Brabander and R. Verbeke, *J. Chromatogr.*, 108 (1975) 141–151.
2 R. Verbeke and H. F. de Brabander, *Method of Analysis for detecting Anti-thyroid Substances in Fresh Muscle Tissue*, Commission of European Communities, Directorate General for Agriculture. Document No. 1123/VI/78-EN, Brussels, 1978.
3 C. H. van Etten and I. A. Wolff, *Toxicants occuring naturally in Foods*, Nat. Acad. Sci., Washington, D.C., 1973, pp. 210–234.
4 M. A. Greer, *Recent Progr. Horm. Res.*, 18 (1962) 187–212.
5 F. L. Austin, C. A. Gent and I. A. Wolff, *Can. J. Chem.*, 46 (1968) 1507–1512.
6 M. A. Greer and J. M. Deeney, *J. Clin. Invest.*, 38 (1959) 1465.
7 E. L. Oginsky, A. E. Stein and M. A. Greer, *Proc. Soc. Exp. Biol. Med.*, 119 (1965) 360.
8 P. Langer, *Hoppe-Seyler's Z. Physiol. Chem.*, 323 (1961) 194.
9 H. F. de Brabander and R. Verbeke, *J. Chromatogr.*, 138 (1977) 131–142.
10 R. Hill, *Brit. Vet. J.*, 135 (1979) 3–16.

11 A. I. Virtanen, M. Kreula and M. Kiesvaara, *Acta Chem. Scand.*, 13 (1959) 1043–1044.
12 M. Peltola, in C. Cassano and M. Andreoli (Editors), *Current Topics of Thyroic Research*, Academic Press, New York, 1965, p. 872.
13 K. Iwarsson, *Acta Vet. Scand.*, 14 (1973) 570–594.
14 K. Iwarsson and P. O. Nelson, *Acta Vet. Scand.*, 14 (1973) 595–609.
15 Y. Akiba and T. Matsumoto, *Tohoku J. Agr. Res.*, 23 (1972) 79–85.
16 Y. Akiba and T. Matsumoto, *Tohoku J. Agr. Res.*, 23 (1972) 86–91.
17 Y. Akiba and T. Matsumoto, *Poult. Sci.*, 55 (1976) 716–719.
18 H. Vogt, *Landwirtsch. Forsch.*, 30 (1973) 210–216.
19 A. W. Pearson and E. J. Butler, *Vet. Rec.*, 104 (1979) 168.
20 A. W. Pearson and E. J. Butler, *Vet. Rec.*, 104 (1979) 318–319.
21 A. W. Pearson, N. M. Greenwood and E. J. Butler, *Vet. Rec.*, 106 (1980) 560.
22 Z. Madjeski, *Med. Weter.*, 30 (1974) 360–363.
23 E. W. Underhill and D. F. Kirkland, *J. Chromatogr.*, 57 (1971) 47–54.
24 W. J. Mullin, *J. Chromatogr.*, 155 (1978) 198–202.
25 M. E. Daxenbichler, G. F. Spencer, R. Kleiman, C. H. van Etten and I. A. Wolff, *Anal. Biochem.*, 38 (1970) 373–382.
26 P. N. Mahaswari, D. W. Stanley, I. J. Gray and F. R. van de Voort, *J. Amer. Oil Chem. Soc.*, 56 (1979) 837–841.
27 Cl. Franzke, R. Gobel, G. Noack and I. Seiffert, *Die Nahrung*, 19, 7 (1975) 583–593.
28 C. G. Youngs and L. R. Wetter, *J. Amer. Oil Chem. Soc.*, 44 (1967) 551–554.
29 H. A. McLeod, G. Benns, D. Lewis and J. F. Lawrence, *J. Chromatogr.*, 157 (1978) 285–290.
30 G. Benns, M. R. L'Abbé and J. F. Lawrence, *J. Agr. Food Chem.*, 27 (1979) 426–428.
31 E. Josefsson and L. Åkerström, *J. Chromatogr.*, 174 (1979) 465–468.
32 C. H. van Etten, M. E. Daxenbichler, W. Schroeder, L. M. Princen and T. W. Perry, *Can. J. Anim. Sci.*, 57 (1977) 75–80.
33 R. Verbeke and H. F. de Brabander, *J. Chromatogr.*, in preparation.
34 M. G. Ettlinger, *J. Amer. Chem. Soc.*, 72 (1950) 4792–4796.
35 F. Wrede, *Die Methoden der Fermentforschung*, Georg Thieme, Leipzig, 1941, Bd 2, p. 1835.
36 K. Blau and G. S. King, *Handbook of Derivatives for Chromatography*, Heyden & Son, London, 1978, p. 132.
37 F. T. Delbeke and M. Debackere, *J. Chromatogr.*, 237 (1982) 344–349.
38 M. Kreula and M. Kiesvaara, *Acta Chem. Scand.*, 13 (1959) 1375–1382.
39 G. L. Baughman, M. H. Carter, N. K. Wolf and R. G. Zepp, *J. Chromatogr.*, 76 (1973) 471–476.