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Note

Determination of thyreostatics in meat by reversed-phase liquid chromatography with ultraviolet and electrochemical detection

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Thyreostatic "anti-hormones" can be used to increase the weight of animals prior to slaughter. The weight increase is due to the increased water content of the gastrointestinal tract and the retention of water in tissue[1]. Meat from animals treated with thyreostatics is subject to exudation and is of inferior quality. Regulations in The Netherlands prohibit the use of anti-hormones in the fattening of cattle[2].

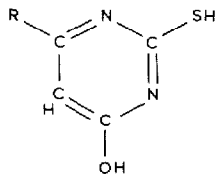
Various techniques have been used for the analysis of thyreostatics such as high-performance thin-layer chromatography[1,3], high-performance liquid chromatography (HPLC) with UV detection[4–7] and histological techniques[8,9]. The use of liquid chromatography with electrochemical detection (LC–ED) of anti-hormones has not yet been reported. An advantage of electrochemical detection is that it is more selective than UV detection in analyses of complex matrices and the signal-to-noise ratio is higher than for UV detection[10–14].

We present a method for the determination of thiouracil (TU) and methylthiouracil (MTU) in meat at the mass fraction level of 10^{-9} (= ppb or μg/kg) by HPLC with UV detection and oxidative electrochemical detection.

EXPERIMENTAL

Chemicals and reagents

All reagents used were analytical grade products. Thiouracil (4-hydroxy-2-mercaptopyrimidine) and methylthiouracil (4-hydroxy-2-mercapto-6-methylpyridimide) were obtained from Fluka.



R = H: thiouracil
R = CH_3: methylthiouracil

Apparatus

The solvent-delivery system (Waters Model 6000 A) was equipped with a pressure gauge as pulse damper. The original pump heads were replaced by Swip pump

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heads (Saphirwerk Industrieprodukte). The advantage of these pump heads is that the plungers can be washed with water to dissolve salts and that the eluent flow is more stable. A Waters Intelligent Sample Processor (WISP) was used as autosampler. A LiChrosorb RP-18 pre-column (10 μm, 30 mm $\times$ 3.2 mm I.D., Brownlee) and a Shandon Hypersil ODS analytical column (5 μm, 250 mm $\times$ 4.6 mm I.D., Chrompack) were used. The mobile phase, 0.01 *M* potassium dihydrogenphosphate–methanol (90:10) with 2 g tetrabutylammonium chloride per litre, was filtered through a 0.45-μm membrane filter with the aid of a solvent clarification kit (Millipore). The flow-rate was adjusted to 1.0 ml/min. All separations were performed at 40°C. The injection volume was 5–200 μl.

The wavelength of the UV detector (Waters Model 450) was 280 nm. Electrochemical detection was achieved with a Metrohm 656 electrochemical detector equipped with a glassy carbon working electrode, a glassy carbon auxilliary electrode and a silver–silver chloride–lithium chloride (3 *M* in water) electrode as reference electrode. The potentiostat was a Metrohm Model VA 641. The potential applied was +1.25 V *vs.* the reference electrode. Chromatographic recordings were made on a dual-pen recorder (Kipp Model BD 41); UV 10 mV, ED 1 V.

Sample pretreatment

A 5-g amount of minced meat was mixed thoroughly with 10 ml of ethyl acetate in a 25-ml test-tube using a Whirlmix. The homogenate was centrifuged for 10 min at a rotational frequency of 1500 min^{-1} (200 *g*). The supernatant was decanted into another test-tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 2.5 ml methylene chloride and transferred to a Baker silica gel (6 ml) cartridge which had been washed with 5 ml acetonitrile–water (20:80), 5 ml acetonitrile and 5 ml methylene chloride. The tube was rinsed with 2.5 ml methylene chloride and the solution was added to the cartridge. The cartridge was washed with 10 ml methylene chloride and after drying it was eluted with 5 ml of acetonitrile–water (20:80). About 10 ml of acetonitrile were added to the eluate and the eluate was evaporated to dryness at 50°C under a stream of nitrogen. The residue was dissolved in 500 μl eluent for liquid chromatography.

RESULTS AND DISCUSSION

Electrochemical operations

The electrochemical pretreatment of the glassy carbon electrode was adapted from that of Engstrom and Strasser[15]. The glassy carbon electrode was pretreated at +1.75 V for 5 min and then at −1.0 V for 1 min. The pretreatment was carried out on-line in the eluent. This pretreatment gave a stable baseline more rapidly in comparison with other methods, like polishing with Alox powder.

An hydrodynamic voltammogram was measured by injecting a solution containing 100 ng of TU and 100 ng MTU. The hydrodynamic voltammogram of TU shows an oxidative wave in the range 600–1300 mV *vs.* the reference electrode (Fig. 1). The hydrodynamic voltammogram of MTU gives almost the same wave. We chose a potential of +1.25 V because this gives the best signal-to-noise ratio.

Fig. 2 shows the chromatograms of a standard solution of 40 ng TU and 40 ng MTU. The electrochemical detector was set to 0.5 μA full scale and the UV

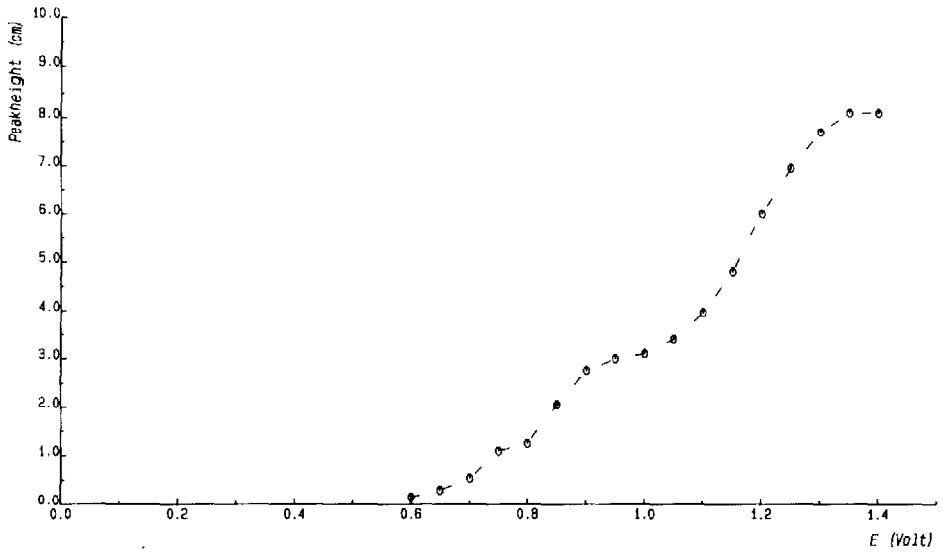

Fig. 1. Hydrodynamic voltammogram of TU. ED: 0.5 μA f.s.

detector operated at the highest sensitivity (0.01 a.u.f.s). In Fig. 3 the results are given for a real sample which contained MTU. The mass fraction of MTU was $8 \cdot 10^{-6}$, *i.e.*, 8 mg/kg or ppm.

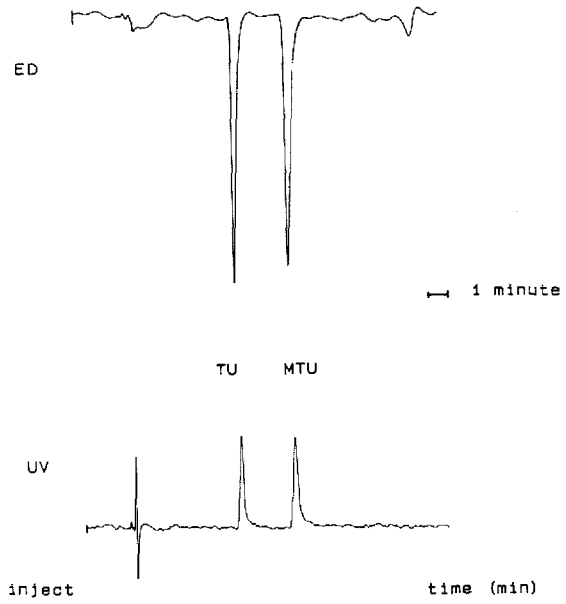

Fig. 2. Chromatogram of a standard solution of 40 ng TU and 40 ng MTU. ED: 0.5 μA f.s. UV: 0.01 a.u.f.s.

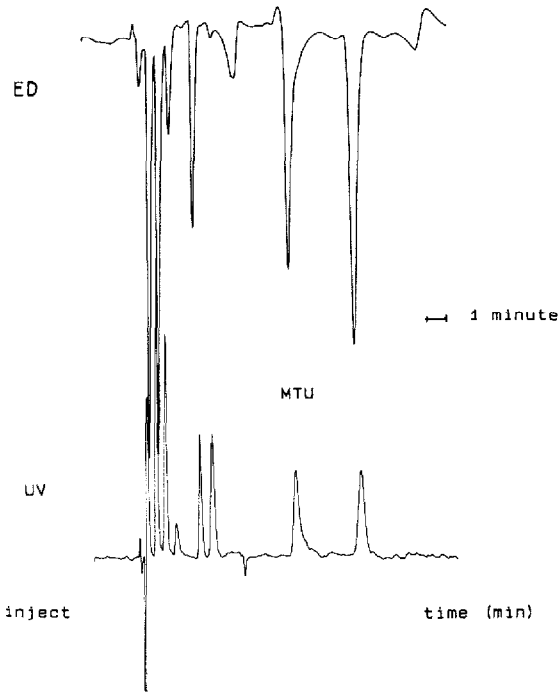

Fig. 3. Chromatogram of a meat sample, containing about 8 μg MTU/g. ED: 5 μA f.s. UV: 0.02 a.u.f.s.

Linear studies

The linearity was evaluated with standard solutions containing various amounts of TU and MTU in the range 4–150 ng for ED and 20–150 ng for UV detection. In the first case the peak heights gave linear relationhips with correlation coefficients of 0.9991 for TU (slope 2.97, *y*-intercept −12.3) and 0.9996 for MTU (slope 3.59, *y*-intercept −11.4). For UV detection the correlation coefficients were 0.997 for TU (slope 0.277, *y*-intercept −0.255) and 0.999 for MTU (slope 0.574, *y*-intercept 0.903).

Recovery experiments

Recovery experiments were performed by adding TU and MTU to meat at concentrations from 100 to 2000 ng/g. The mean recovery (± S.D.) for TU was 66 ± 8% and for MTU was 70 ± 11% (n = 5).

CONCLUSIONS

In analyses of prohibited drugs there is always a risk of false negative or false positive results. To prevent such false positive results, it is advisable to apply two detection methods which are based on different physico-chemical principles. The described combination of ultraviolet detection and electrochemical detection after HPLC separation can fulfil this requirement.

We applied this method to determine MTU in meat samples. Both UV detection as ED gave positive results comparable with the thin-layer chromatography method described by De Brabander[1]. Starting with a portion of 5 g of meat, the limit of detection can be estimated as a mass fraction of $2.5 \cdot 10^{-8}$ (25 μg/kg) for UV detection and of 10^{-8} (10 μg/kg) for ED.

The very simple sample pretreatment using commercial silica gel columns was a sufficient clean-up procedure.

With the proposed method it is possible to determine TU and MTU in meat.

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