Determination of Thyreostatic Drugs by HPTLC with Confirmation by GC-MS

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Key Words:
HPTLC
GC-MS
Thyreostatic drugs

Summary

A HPTLC method has been developed for the qualitative and quantitative determination of residues of thyreostatic drugs in biological material. Clean-up is performed with a rapid and selective extraction procedure based on formation of a specific complex between the drugs and mercury ions bound to an affinity column, and the determination is based on the fluorescence induction of the 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole derivatives of the drugs with cysteine after HPTLC separation. Additional confirmation of the identities of suspect material in spots of the correct Rf value may be obtained by GC-MS with positive chemical ionization.

1 Introduction

The use of the thyreostatic drugs for promoting animal growth is prohibited in all EEC member states. In contrast with the lack of consensus on anabolic agents, there is general agreement on the ban of these drugs: not only may thyreostatic drugs be harmful to human health but the meat derived from animals treated with the drugs may be of inferior quality. The weight gain obtained with thyreostats consists mainly of increased filling of the gastrointestinal tract and higher water retention by the animal. In Belgium, control of the illicit application of growth promoters is performed according to EEC directives [1,2] by the Institute of Veterinary Inspection (IVK-IEV) and the Ministry of Agriculture.

Treatment of cattle with thyreostatic drugs may be detected from the presence of residues in the plasma, excreta, meat, or organs of the animal. In regulatory control at the farm, plasma, urine, and/or feces of the animals may be sampled. At the retail level (butcher’s shop, supermarket) or for import/export control, sampling is restricted to tissue only. At the slaughterhouse, tissue as well as excreta can be sampled and, moreover, inspection of the weight of the thyroid gland may give an indication of the possible administration of antithyroid drugs. Finally, all kinds of matrices (powders, fluids, fodder) circulating on the (black) market have to be analyzed for the presence of thyreostatic drugs.

The most important and powerful thyreostatic drugs presently in use are thioracil (TU) and analogous compounds (especially methylthiouracil (MTU) and tapazole (TAP), Figure 1). Specific procedures for the detection of this group of drugs have already been described [3-5]; the methods are based on the fluorescence induction of the NBD-Cl (7-chloro-nitrobenzo-2-oxa-1,3-diazole) derivatives of the drugs with cysteine, combined with a rapid and selective extraction procedure based on the formation of specific complexes of the drugs with mercury ions. These methods have been adopted by the Benelux countries [6] and the EEC [7] for the qualitative analysis of these drugs at the 50 ppb level. Sample clean-up with the mercurified affinity column was also used by Schilt [8] in a GC-NPD method.

Because access to most of the literature cited above [4-7] is restricted, and since, because control of anabolic agents has been strengthened in recent years, there is a risk that the use of thyreostatic drugs will increase, we have investigated the most recent modifications of the NBD-Cl method. Scientific details and some quantitative results are presented here. In order to improve the qualitative accuracy of the method, confirmation of the identity of suspect material in spots with the correct Rf values has been provided by GC-MS analysis using a Finnigan Mat ITS40 mass spectrometer with positive chemical ionization.

Figure 1


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2 Experimental

2.1 Reagents and Standard Solutions

2.1.1 Reagents

Buffer of pH 8 was prepared from a mixture of disodium hydrogen phosphate (0.2 M, 94.5 ml) and potassium dihydrogen phosphate (0.2 M, 5.5 ml). The pH is monitored and eventually adjusted to 8.

NBD-CI solution (25 μmoles/ml) was prepared by dissolving NBD-CI (5 mg) in methanol (1 ml). The solution was prepared fresh daily and stored in a cool, dark place.

2.1.2 Spray Solutions

Solution I was prepared by mixing denatured ethanol (50 ml) with propan-2-ol (50 ml) and adding 25 % ammonia (2 ml).

Solution II was prepared by dissolving cysteine hydrochloride (0.6 g) in water (20 ml).

Immediately before use solution I (100 ml) and solution II (2 ml) were mixed.

2.1.3 Standard Solutions

A stock solution of the thyoestatic drugs (TU, MTU, propylthiouracil (PTU), phenylthiouracil (PhTU), and TAP; 20 mg/100 ml or 200 ng/μl) was prepared in methanol. A working solution was obtained by 100-fold dilution with methanol (2 ng/μl).

Internal standard solution (200 ng/μl) was prepared by dissolving DMTU (45.6-dimethyl-2-thiouracil; 20 mg) in methanol (100 ml). A working solution (2 ng/μl) was obtained by 100-fold dilution with methanol.

2.2 Preparation of the Mercurated Resin

Dowex 1×2 (50-100 mesh, analytical grade) was washed successively with 10 bed volumes of distilled water, 0.5 N sodium hydroxide solution, distilled water, 0.5 N acetic acid, and distilled water. The wet anion exchanger (10 ml) was shaken with an aqueous solution of 2,7-dibromo-4-hydroxy-mercurifluorescein (250 mg/100 ml water) for 24 h. The mercurated resin was washed with water until the eluate was colorless, treated with hydrochloric acid (0.1 N) in sodium chloride solution (0.5 M, 100 ml), washed with distilled water (500 ml), treated with sodium hydroxide solution (0.1 N, 100 ml), and, finally, washed with distilled water (500 ml). The mercurated resin was stored in the dark.

2.3 Micro Column for Cleaning Extracts of Thyroestatic Drugs

A diagram of the chromatographic micro column, used in the 1980s for cleaning extracts of thyoestatic drugs, is given in Figure 2. A detailed description is given elsewhere [5]. Alternatively, columns designed for immunoaffinity chromatography (e.g. Bio-rad Econo columns) or solid phase columns may be used.

![Diagram of micro column](image)

Figure 2

Micro affinity column for clean-up of thyroestatic drugs: 1, glass column (4 mm i.d., 6 mm o.d.); 2, glass rod (8 mm o.d.); 3, silicone rubber tubing (4 mm i.d., 6 mm o.d.); 4, silicone rubber tubing (0.5 mm i.d., 4 mm o.d.); 5, silicone rubber tubing (0.5 mm i.d., 1 mm o.d.); 6, Teflon tubing (0.3 mm i.d., 0.7 mm o.d.); 7, glass funnel.

The column is filled with water and the glass rod removed. Mercurated resin (ca 0.6 ml) is suspended in water and added to the glass funnel. After sedimentation of the resin in the glass column to a height of 5 cm, excess resin is removed and, after the glass rod had been replaced so that it is resting on the resin bed, the column is ready for use. The final column volume is ca. 50 mm x 4 mm (0.6 ml).

2.4 Analytical Procedure

2.4.1 General Procedure

Tissue (2 g), or urine, plasma, or skimmed milk (2 ml) were homogenized in methanol (10 ml) in 10-20 ml flasks by means of an Ultra-Turrax. Internal standard solution (DMTU, 100 μl, equivalent to 100 ppb) was added and the homogenate centrifuged (e.g. Sorvall) at 10900 rpm (12000 g) for 10 min. The supernatant was decanted and allowed to percolate through the mercury column which was then washed with distilled water and the thyoestatic drugs eluted with a solution of hydrochloric acid (0.1 N) in sodium chloride solution (0.5 M, 5 ml, pH 1). Buffer solution (pH 8, 1 ml) is added to the eluate.

The eluate was then neutralized and adjusted to pH 8, methanolic NBD-CI solution (0.1 ml) added, and the reaction allowed to proceed in the dark at 40 °C (water bath) for 1 h. Thereafter, the reaction mixture was adjusted to pH 3-4 by...
addition of hydrochloric acid (6 N, 0.2 ml) and the NBD derivatives extracted with diethyl ether (1 x 3 ml + 2 x 2 ml). The combined ether extracts were dried over sodium sulfate and concentrated, under a jet of nitrogen (e.g. Techni Dry Block) to a final volume of 0.1-1 ml, depending on the concentration range under investigation.

2.4.2 Short Procedure for Urine
Urine (0.1-0.5 ml) was mixed with buffer solution (pH 8, 5 ml) and internal standard (100 µl, equivalent to 0.4-2 ppm). The solution was derivatized with NBD-Cl and extracted as described above.

2.4.3 Short Procedure for Solid Matrixes (e.g. Fodder)
Powder or fodder (≤ 2 g) was mixed with methanol (4 ml) in an ultrasonic bath. After centrifugation at 10000 rpm (12000 g) for 10 min the supernatant was mixed with buffer solution (pH 8, 5 ml), and derivatized with NBD-Cl and extracted as described above [11].

2.4.4 Derivatized Standard Solutions for TLC
Stock solution (0.1 ml, equivalent to 20 µg) was mixed with buffer solution (pH 8, 5 ml) and derivatization reagent. After derivatization and extraction the volume of the ether extract was reduced to 1 ml, yielding a 20 ng/µl solution.

2.4.5 Derivatized Standard Solutions for GC-MS
A standard mixture of thyrostatic drugs (20 ng/µl) was prepared from the standard solutions and 10 µl of this solution transferred to an autosampler vial, evaporated to dryness, and reacted (15 min at 60 °C) with MSTFA (50 µl).

2.5 Chromatography
The concentrated extract (5-10 µl of 0.1-1 ml µl) was applied to an HPTLC plate by means of a sample applicator designed in-house (described elsewhere [5,9]). A "4 x 4" sample applicator and the faster "4 x 4" developing mode can be used as an alternative [10,12]. Derivatized standard solutions (1 µl) were applied to the side lanes of the plates.

2.5.1 Qualitative Determination
The extracts were analyzed by two-dimensional chromatography on 10 x 10 cm or 5 x 5 cm HPTLC plates precoated with silica gel 60 (Merck); 20 x 20 cm TLC plates can also be used but the application volumes have to be adjusted accordingly.

Development was performed in unsaturated tanks to a distance of 3-4 cm using dichloromethane – methanol (98 + 2, v/v) as mobile phase. The plate was dried in air and the origin of the sample over-spotted with 20 ng of the thyrostatic drug assumed to be present. This procedure was useful for additional Rf comparison between an unknown spot and the reference after the second development. The plate was then turned through 90° and developed in the second direction with dichloromethane – propionic acid (98 + 2, v/v).

In "4 x 4" developing mode 4 samples were developed in two dimensions on one HPTLC plate [12].

After drying of the plates, fluorescence was induced (by spraying or dipping) with the alkaline cystine solution described in Section 2.1.1 and illumination with UV light (λ = 366 nm with contrast filter). The spots were identified by comparison of their Rf values with those measured for the derivatized standard solutions.

2.5.2 Quantitative Determination
The relative fluorescence intensities of the thyrostatic drug derivatives were measured against the derivative of the internal standard (DMTU) by densitometric scanning in the fluorescence mode (KM 3 TLC Scanner; Zeiss, Oberkochen, FRG).

2.6 GC-MS Confirmation
GC-MS analysis was performed with an IT-S40 ion Trap Mass Spectrometer (Finnigan MAT, San Jose, CA, USA) fitted with a 30 m x 0.25 mm i.d. column coated with a 0.25 µm film of DB-5 (J&W).

The spot suspected of containing the drug was removed from the plate and transferred to an autosampler vial (100 µl). MSTFA (25 µl) was added, the contents mixed, and the vial heated to 100 °C for 15 min. After sedimentation of the silica gel, 1-2 µl of the clear supernatant was analyzed by GC-MS.

The oven temperature for (splitless) injection was 100 °C; this was maintained for 2 min, programmed at 1 min/min to 150 °C, and then at 20 °C/min to 250 °C, which was held for 3 min (total program 20 min).

Positive chemical ionization spectra (reagent gas, isobutane) between m/z 90 and 400 were acquired for 20 min at 1 scan/s (filament, multiplier delay 800 s). The retention times and the ions used for tracing the peaks after full-scan acquisition are summarized in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>T1</th>
<th>Ion 1</th>
<th>Ion 2</th>
<th>Ion 3 (MH^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP</td>
<td>13.2</td>
<td>171</td>
<td>186</td>
<td>259</td>
</tr>
<tr>
<td>TU</td>
<td>13.4</td>
<td>257</td>
<td>273</td>
<td>345</td>
</tr>
<tr>
<td>MTU</td>
<td>14.3</td>
<td>271</td>
<td>287</td>
<td>359</td>
</tr>
<tr>
<td>DMTU</td>
<td>15.2</td>
<td>285</td>
<td>301</td>
<td>373</td>
</tr>
<tr>
<td>PTU</td>
<td>15.6</td>
<td>299</td>
<td>315</td>
<td>387</td>
</tr>
</tbody>
</table>

<sup>a</sup> indicative

The standard mixture prepared for GC-MS (1 µl, equivalent to 4 ng) was analyzed as described above. Identification of unknown compounds was performed by comparison of full-scan spectra with those in a library compiled in-house.
3 Results and Discussion

3.1 Mercured Affinity Column

The extension of our first clean-up procedure [3] to quantitative HPTLC was not successful: it was found that the recoveries of thyreostatic drugs added to samples were significantly lower than those recovered from standard solutions. Recoveries were reproducible within the same sample but extremely variable between samples (especially within urine samples). Biological material obviously contains unknown products in variable concentrations which may inhibit the reaction of NBD-Cl with the thyreostatic drugs. This is illustrated in Figure 3 by the decrease in extraction yield of identical amounts of 14C-thiouracil added to increasing amounts of urine (the determination was performed by means of a Tri Carb liquid scintillation counter (Packard, La Grange, Ill., USA)).

A new clean-up procedure was, therefore, sought as a means of overcoming this problem. The name mercaptan (an old name for thiol) is derived from the observation that thiol groups form very specific complexes with mercury. Several attempts were made to use this specific complex formation for a clean-up and it was found that strong anion-exchangers (e.g. Dowex 1) strongly bind mercurial dyes (e.g. 2,7-dibromo-4-hydroxymercuriflouruscin (DBMF)). The strong bond between a mercurial dye and Dowex-1 has already been described by Zak [13]: neither strong acids (e.g. 0.5 M hydrochloric acid) nor strong bases (e.g. 0.5 M sodium hydroxide solution) are capable of displacing the dye from the resin.

The testing of the binding characteristics of DBMF with Dowex-1 resins with various types of cross-linkage has already been described [5]: Dowex 1 x 2 was selected for clean-up of thyreostatic drugs. This resin binds 25 mg DBMF/ml resin, equivalent to 6.7 mg (33 µmoles) Hg(ll)/ml wet resin. This binding capacity is 2500 (PTU) to 2700 (TU) µg thyreostatic drug. Hitherto the highest concentration ever found in a thyroid gland was 200 ppm (400 µg/2 g sample), so the binding capacity may be considered sufficiently high.

3.2 Clean-up of Thyreostatic Drugs on DBMF Columns

The adsorption of thiouracil on DBMF columns was studied using 14C-thiouracil. After washing the column with distilled water the drug was eluted with an acid salt (0.5 M sodium chloride) solution. The elution yields of TU at different pH values, as a function of the elution volume, are given in Figure 4. A salt solution, with a pH of 1 results in optimum elution of the thiouracil from the column.

![Figure 4](image)

Elution yield [%] vs. Volume of urine [ml]

**Figure 3**
Reduction in the extraction yield of 14C-thiouracil with increasing amount of urine.

The adsorption and elution yields of TU and other thyreostatic drugs are summarized in Table 2. The adsorption of the drugs from a methanol – water (80 + 20, v/v) extract (10 ml) on micro DBMF columns (0.6 ml) is practically quantitative. Most of the thyreostatic drugs studied (TU, MTU, PTU, DMTU) were recovered in a 80% yield by elution with 5 ml of a solution of a pH 1 hydrochloric acid (0.1 n) in sodium chloride solution (0.5 M).

Recoveries were lower both for PhTU, perhaps because interaction of the phenyl group with the polystyrene matrix of the resin leads to strong adsorption of this compound, and for TAP, the recovery of which was 60%.

<table>
<thead>
<tr>
<th></th>
<th>TU</th>
<th>MTU</th>
<th>PTU</th>
<th>DMTU</th>
<th>PHTU</th>
<th>TAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not adsorbed [%]</td>
<td>3.8</td>
<td>0.5</td>
<td>15</td>
<td>0.5</td>
<td>8.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Elution yield:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ml [%]</td>
<td>82.0</td>
<td>80.0</td>
<td>78.0</td>
<td>79.0</td>
<td>17.0</td>
<td>60(a)</td>
</tr>
<tr>
<td>10 ml [%]</td>
<td>96.0</td>
<td>92.0</td>
<td>94.0</td>
<td>95.0</td>
<td>24.0</td>
<td>80(a)</td>
</tr>
</tbody>
</table>

\(a\) partially oxidized
Table 3
Reproducibility of thyreostatic drug (TU) analysis in meat.

<table>
<thead>
<tr>
<th>Step</th>
<th>n(^d)</th>
<th>Mean ± SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column elution</td>
<td>26</td>
<td>81 ± 3.9</td>
<td>± 4.8</td>
</tr>
<tr>
<td>Derivatization</td>
<td>26</td>
<td>76 ± 5.0</td>
<td>± 6.6</td>
</tr>
<tr>
<td>HPTLC</td>
<td>22</td>
<td>68 ± 4.7</td>
<td>± 5.4</td>
</tr>
<tr>
<td>Total procedure</td>
<td>22</td>
<td>55 ± 6.5</td>
<td>± 12.6</td>
</tr>
</tbody>
</table>

\(^d\) number of determinations

The column was observed to cause oxidation of TAP, to an extent which varied with the batch of Dowex-1 used: although the degree of oxidation was reduced by use of analytical grade material, the exact reason for the oxidation is still unknown. This phenomenon restricts the quantitative analysis of thyreostatic drugs to TU, MTU, and PTU.

3.3 Recovery and Reproducibility

The recovery and reproducibility of the determination of TU in meat, measured using \(^{14}\)C-thiouracil, is given in Table 3. In each step a yield of ca 80% was obtained giving a total recovery of 55%. The coefficient of variation of the total procedure was 13%. The recovery of MTU, TU, and PTU, at the 100 ppb level, from meat, plasma, and milk using DMTU as internal standard is given in Table 4. Recoveries from meat were quantitative for the drugs studied; those from plasma and milk were quantitative for MTU but substantially lower for PTU and TU.

Table 4
Recovery of thyreostatic drugs from various media using the internal standard (DMTU) procedure.

<table>
<thead>
<tr>
<th>Biological material</th>
<th>Concentration added [ppb]</th>
<th>Concentration found ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTU</td>
<td>MTU</td>
</tr>
<tr>
<td>Meat (3(^d))</td>
<td>100</td>
<td>106 ± 5.5</td>
</tr>
<tr>
<td>Plasma (3(^d))</td>
<td>100</td>
<td>84 ± 6.2</td>
</tr>
<tr>
<td>Milk (3(^d))</td>
<td>100</td>
<td>88 ± 6.9</td>
</tr>
</tbody>
</table>

\(^d\) number of determinations

3.4 GC-MS Confirmation

Because, according to the Belgian law a so-called “positive” result on one sample, obtained in one laboratory may be (and often is) challenged by the owner of the animal, a second analysis, in a second independent laboratory, should also be performed. Discussions between different countries about the reliability of the results of residue analysis also arise in connection with the import/export of meat. In contrast with the fluorescence induced in steroids, from which all kinds of colors are generated, all the NBD derivatives of the thyreostatic drugs are yellow. To improve the qualitative accuracy of the analysis, and to prove the identity with more analytical security, the HPTLC method was combined with GC-MS in order to obtain a mass spectrum of suspect material in spots with the correct \(R_f\) values.

Thyreostatic drugs could be derivatized with different reagents; MSTFA was tried first because it was also used for the derivatization of steroids. It was found that the most important of the drugs were easily derivatized with MSTFA, forming the di-TMS (TAP) and tri-TMS (TU, MTU, DMTU, and PTU) derivatives, all of which could be separated on an apolar column (e.g. DB-5) as is illustrated in Figure 5. The chemical ionization mass spectra of all the drugs contain at least 2 characteristic ions together with the pseudo-molecular ion; these ions are listed in Table 1.

Figure 5
Gas chromatogram of TMS derivatives of thyreostatic drugs (standard solution).

Figure 6
Gas chromatogram of MTU standard from HPTLC plate, with corresponding mass spectrum.
It was also found that the identity of a spot (corresponding to a thyreostatic drug) on a HPTLC plate could be confirmed by scratching the spot from the plate after fluorescence induction, transferring the silica gel to an autosampler vial and derivatizing the contents with MSTFA.

The temperature needed to derivatize the material in the spots was higher than that needed for the standards. This is not surprising since the drug-NBD derivatives are converted to cysteine-NBD derivatives for fluorescence induction, liberating the free thyreostatic drugs once more on to the plate. A GC-MS total ion chromatogram and a mass spectrum of MTU obtained from a spot on an HPTLC plate are shown in Figure 6. As can be seen, the chromatogram contains a considerable number of peaks, owing to the background from the plate. The peak corresponding to MTU is separated from the background and a library searchable mass spectrum can be obtained. There is, however, a tendency for the pseudo-molecular ion to disappear at lower concentrations.

3.5 MTU Concentrations in Organs and Muscular Tissues

The concentration of MTU was determined in the thyroid, kidney, and muscle of five animals obtained from regulatory control; the results are summarized in Table 5. As expected, the highest concentration of MTU was found in the thyroid; in this organ the concentration of MTU was 20-100 times greater than the mean concentration found in muscle (0.2-1.9 ppm). The concentration of MTU in the kidney was significantly higher ($p < 0.005$) than that in muscular tissues and a significant difference ($p < 0.005$) was found between the MTU content of the muscles M. Psoas (P), the diaphragm (D), and M. Trapezius (T) (non-parametric test of Wilcoxon). Classification of the different tissues in descending order of MTU concentration yields:

thyroid > kidney > P = D = LD > C > S > G > T

Table 5

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>30.60</td>
<td>48.00</td>
<td>53.20</td>
<td>41.50</td>
<td>37.50</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.30</td>
<td>0.81</td>
<td>2.1</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>M. Long. Dorsi (LD)</td>
<td>0.16</td>
<td>0.51</td>
<td>1.0</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>M. Psoas (P)</td>
<td>0.21</td>
<td>0.63</td>
<td>1.7</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Cervical Muscle (C)</td>
<td>0.22</td>
<td>0.40</td>
<td>1.2</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>M. Gastrocnemius (G)</td>
<td>0.15</td>
<td>0.79</td>
<td>1.8</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>M. Trapezius (T)</td>
<td>0.17</td>
<td>0.54</td>
<td>1.0</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>M. Solius (S)</td>
<td>0.18</td>
<td>0.46</td>
<td>3.0</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Diaphragm (D)</td>
<td>0.19</td>
<td>0.59</td>
<td>1.7</td>
<td>2.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

seperated and each analyzed for their MTU content. Four different muscles have been analyzed using the procedure described: typical results are presented in Figure 7. Our measurements show that MTU is not substantially degraded by prolonged heating of the meat. Since only 25% of the total content is recovered in the dripping (= 35% of the muscle weight), MTU residues are concentrated in the cooked meat.

4 Conclusion

In comparison with previous clean-up procedures, mercurated affinity columns have several advantages which enable quantitative analysis of MTU, TU, and PTU and the study of residue concentrations of these drugs in slaughter animals. Such data may be valuable for veterinary inspection. The partial oxidation of TAP on the mercury column has, unfortunately, so far inhibited the quantitative analysis of this drug.

In beef carcasses obtained from regulatory control the levels of residues in the thyroid were 20-100 times higher than in the muscular tissues. Cooking experiments demonstrated that MTU residues in meat are not substantially destroyed by heating.

The speed of this clean-up procedure, in combination with HPTLC and specific fluorescence detection is important for the qualitative analysis needed for routine control in the EEC: the speed and selectivity of the analysis is improved and the final extracts are clearer and thus easier to evaluate. In addition to MTU, TU, and PTU the method could also be used for...
routine control of tapazole but with a higher detection limit (100 ppb instead of 25 ppb).

The coupling of the HPTLC method with GC-MS improves the qualitative accuracy of the analysis. The identity of the residue follows from two $R_f$ values on a HPTLC plate, one retention time on a capillary column, and a full-scan mass spectrum, thus fulfilling quality criteria for residue analysis [14]. Such coupling, moreover, also enables mono-dimensional HPTLC to be used as a screening method without the need for 2D-HPTLC: suspect spots could be analyzed directly by GC-MS. For routine control at the farm, the short procedure for urine with direct derivatization, coupled with GC-MS, can be very useful.

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References


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