ELSEVIER

Journal of Chromatography A 819 (1998) 99–111

JOURNAL OF CHROMATOGRAPHY A

Confirmation of residues of thyreostatic drugs in thyroid glands by multiple mass spectrometry after thin-layer chromatographic screening

K. De Wasch[a,*], H.F. De Brabander[a], L.A. van Ginkel[b], A. Spaan[b], S.S. Sterk[b], H.D. Meiring[c]

[a]*Faculty of Veterinary Medicine of the University of Ghent, Department of Veterinary Food Inspection, Laboratory of Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium*

[b]*RIVM: European Union Community Reference Laboratory, A. Van Leeuwenhoeklaan 9, P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands*

[c]*RIVM: Laboratory for Organic Analytical Chemistry, A. Van Leeuwenhoeklaan 9, P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands*

Abstract

A method is described for the confirmation of high-performance thin layer chromatography (HPTLC) suspect results of residues of thyreostatic drugs in thyroid tissue. The method is based on the infusion of the remainder of the extract used for HPTLC via the electrospray interface into a mass spectrometer operating in the multiple stage mass spectrometry (MS^n) mode. The clean-up of the samples was performed with a selective extraction procedure, based on a specific complex formation of the drugs with mercury ions bound in an affinity column. The thyreostatic drugs were derivatised with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

Keywords: Thyreostatic drugs; HPTLC; Multiple mass spectrometry

1. Introduction

When thyreostatic drugs are given to animals, the decreased production of thyroid hormones reduces the basal metabolism, lowers gastro-intestinal motility and favours extracellular water retention. Therefore the mass gain obtained with thyreostats consists mainly of an increased filling of the gastro-intestinal tract and an increased water retention by the animal. In contradiction to some anabolic agents such as the natural hormones, there is a world-wide agreement on the ban of these drugs: thyreostatic drugs may be harmful to human health, the consumer is mislead (sold water for the price of meat) and the quality of the meat of animals treated with the drugs may be inferior.

Screening of cattle for possible treatment with thyreostatic drugs may be based on morphological properties (macroscopical properties due to the hypothyroid status, histological examinations). The inspection of the thyroid gland (e.g., the mass of the gland) may give an indication of the possible administration of anti-thyroid drugs. However, the relation between the mass of the thyroid, used for screening purposes by many European countries and

*Corresponding author.

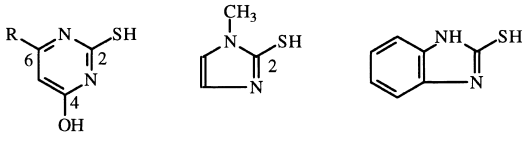
0021-9673/98/$19.00
PII: S0021-9673(98)00463-4

the treatment with thyreostats is not unequivocal. Small thyroids can also contain residues of thyreostats. The confirmation of these screening tests is given by chemical analysis of the residues present in plasma, excreta, meat or organs of the animal. The highest concentration of thyreostatic drugs is present in the thyroid gland.

In regulatory control at the farm, plasma, urine and/or faeces may be sampled. At the retail level (butcher shop, supermarket) or in the case of import/export, sampling is restricted to tissue only. At the slaughterhouse, tissue as well as excretia, can be sampled. Finally, all kinds of other matrices such as powders and fluids circulating on the (black) market have to be analysed for the presence of thyreostatic drugs.

The most important and powerful thyreostatic drugs, hitherto used are thiouracil and analogous compounds [specially methylthiouracil (MTU)] and tapazole (TAP). Recently, new compounds as mercaptobenzimidazole (MBI) are said to have been misused in some countries of the European Union (EU). In Fig. 1 the most important formulae of these drugs are given.

Procedures for the detection of these drugs in biological material have been described a long time ago by our laboratory [1,2]. These methods are based on the fluorescence induction of the NBD derivatives (NBD-Cl=7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) of the drugs with cysteine, combined with a rapid and selective extraction procedure, based on a specific complex formation of the drugs with mercury ions. These methods were adopted by the BENELUX (Belgium, Netherlands and Luxemburg) and the EU for qualitative analysis of these drugs at the 50 ppb level. At that time (1975) high-performance thin-layer chromatography (HPTLC) with fluorescence detection was the only affordable analytical technique for obtaining low detection limits in residue analysis in our laboratory. Later on the HPTLC results could be confirmed with gas chromatography–mass spectrometry (GC–MS) [3] by scraping of the suspect spot from the plate and injecting it, after derivatisation, into the GC system. This procedure increased the selectivity of the identification power of the method. Direct GC–MS methods for the determination of thyreostats in urine were also described [4].



I : 4(6)-R-2-thiouracil **II** : tapazole **III:** mercaptobenzimidazole

Fig. 1. Structural formulae of thyreostatic drugs. **I**: 4(6)-R-Thiouracil (R=H, methyl, *n*-propyl or phenyl) **II**: 1-methyl-2-mercaptoimidazole (tapazole) **III**: MBI: mercaptobenzimidazole.

The sample clean-up with the mercurated affinity column was also used by Schilt [5] in a GC–nitrogen phosphorous detection (NPD) method. Buhlert [6] described the use of GC–MS after derivatisation of the thyreostats with pentafluorobenzylbromide. Recently, modern methods for the analysis of thyreostats in biological material using GC–MS [7–9] were described by the French National Reference Laboratory, Nantes. A LC–MS method [10] was described by a Northern Ireland group. With these methods low limits of detection (LODs) could be reached. However, in order to maintain a good sample throughput of a routine laboratory, screening methods should be spread over all the available equipment of the laboratory. Otherwise, samples may tend to queue for a particular apparatus.

In this investigation the most recent modifications of the NBD-Cl method are presented. Recent advances in ion trap technology have resulted in the possibility of multiple mass spectrometry.

In order to improve the qualitative support of the method, the samples that were suspect during screening with HPTLC were subjected to a confirmatory method by infusion of the remainder of the extract into an ion trap mass spectrometer operating in multiple stage mass spectometry (MS^n) mode (Finnigan MAT LCQ). Ionisation was performed by the electrospray interface and fragment ions were acquired up to MS^3.

2. Experimental

2.1. Apparatus

The following apparatus was used: homogeniser (e.g., Ultra-turrax; Janke and Kunkel, Staufen, Ger-

many), centrifuge Sorvall RC-26 plus (DuPont, Newton, CT, USA), water bath (GFL, Burgwedel, Germany), extraction flasks (10–20 ml), nitrogen evaporator, chromatographic columns and tanks, UV source of 366 nm with contrast filter, sample applicator (e.g., laboratory-made single [11] or '4×4' sample applicator [12]).

The mass spectrometer and the data system used were a LCQ Ion Trap mass analyser (Finnigan MAT, San Jose, CA, USA), with an electrospray interface and Navigator 1.0 software, respectively.

The instrument was operated in positive ion mode. The maximum injection time was set at 400 ms with a total of 2 microscans per scan. An electrospray voltage of 4.5 kV and a capillary temperature of 220°C were used. For the direct infusion of the samples a built-in syringe pump of the LCQ was used.

2.2. Reagents and standard solutions

2.2.1. Reagents

Na_2HPO_4, KH_2PO_4, propan-2-ol, 25% ammonia, L-cysteine hydrochloride, NaCl and HCl were obtained from Merck (Darmstadt, Germany). NBD-Cl was obtained from Sigma–Aldrich (St. Louis, MO, USA).

Buffer pH 8: 94.5 ml of 0.2 *M* Na_2HPO_4 was mixed with 5.5 ml of 0.2 *M* KH_2PO_4. The pH was controlled and adjusted to 8.

NBD-Cl solution (12.5 μ*M*): 5 mg of NBD-Cl was dissolved in 2 ml of methanol. The solution was prepared fresh daily and kept in a cool dark place.

HPTLC plates were obtained from Merck (No. 5547) (Darmstadt, Germany).

Spray solutions: I: 50 ml of denaturated ethanol was mixed with 50 ml of propan-2-ol. 2 ml of 25% ammonia was added. II: 0.24 g of L-cysteine hydrochloride was dissolved in 4 ml of distilled water.

Solution II was mixed immediately before use with solution I.

2.2.2. Standard solutions

Standards were obtained from Sigma–Aldrich. Stock solution of the thyreostatic drugs [2-thiouracil (TU), 6-methyl-2-thiouracil (MTU), 6-propyl-2-thiouracil (PTU), 6-phenyl-2-thiouracil (PhTU), 2-mercaptoimidazole (tapazole, TAP) and 2-mercaptobenzimidazole (MBI)] were prepared in methanol at a concentration of 20 mg (100 ml)$^{-1}$ (200 ng μl^{-1}). A working solution was obtained by 200× dilution in methanol (1 ng μl^{-1}).

Internal standard solution: 20 mg DMTU [4(5,6)-dimethyl-2-thiouracil] was dissolved in 200 ml methanol (200 ng μl^{-1}). A working solution was obtained by 200× dilution in methanol (1 ng μl^{-1}).

2.3. Preparation of the mercurated resin

Dowex 1-X2 (50–100 mesh, analytical grade) obtained from Serva (Heidelberg, Germany) was washed successively with ten bed volumes distilled water, 0.5 *M* NaOH, distilled water, 0.5 *M* acetic acid and distilled water. The wet anion exchanger (10 ml) was shaken with an aqueous solution of 2,7-dibromo-4-hydroxymercurifluorescein (250 mg dissolved in 100 ml water) during 24 h. The mercurated resin was washed with water until the eluate was colourless. Afterwards the resin was treated with 100 ml of 0.1 *M* HCl in 0.5 *M* NaCl, washed with 500 ml distilled water, treated with 100 ml of 0.1 *M* NaOH and finally washed with 500 ml distilled water. The mercurated resin was stored in the dark.

2.4. Microcolumn for the clean-up of thyreostatic drugs

The chromatographic microcolumn, used in the 1980s for the clean-up of thyreostatic drugs, was replaced by a more practical, disposable one. By this change mercurated columns may be used in a standard solid-phase extraction (SPE) system. A scheme is shown in Fig. 2. Alternatively other disposable columns may be used.

2.5. Analytical procedures

General procedure: 2 g of thyroid tissue were homogenised in 10 ml of methanol using an ultra-turrax. The internal standard solution (DMTU, 500 μl, equivalent to 250 ppb) was added. After homogenisation the mixture was centrifuged at 10 000 rpm (12 000 *g*) during 20 min. A disposable column (Fig. 2) was equipped with a frit and filled with 1 ml of

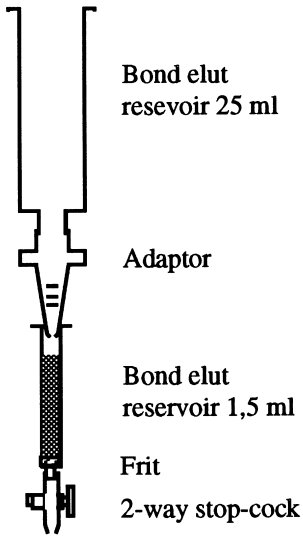


Fig. 2. Microaffinity column for clean-up of thyreostatic drugs.

mercurated resin. The column and reservoir were placed on a SPE vacuum system. The supernatant was decanted and percolated through the mercury column. The column was washed with distilled water and the thyreostatic drugs were eluted with 5 ml elution solution (0.5 *M* NaCl; 0.1 *M* HCl; pH=1). One ml of buffer pH 8 was added and the eluate was neutralised and adjusted to pH 8. A methanolic NBD-Cl solution (0.1 ml) was added and the reaction allowed to proceed in the dark at 40°C during 1 h. Thereafter, the reaction mixture was adjusted to pH 3–4 by adding 0.2 ml 6 *M* HCl. The NBD derivatives were then extracted with successively 3, 2 and 2 ml diethyl ether. The combined ether extracts were dried over sodium sulphate and concentrated under a stream of nitrogen, according to the concentration range investigated, to a volume of 50–100 μl.

2.6. HPTLC

The extracts were analysed by two-dimensional chromatography on precoated silica gel 60 nanoplates (HPTLC plates:10×10 cm or 5×5 cm). Development was carried out in non-saturated tanks. At the starting point of the nanoplates up to 10 μl of the extract was spotted. Appropriate concentrations of the reference mixtures were applied in the side lanes. Chromatographic development was carried out over a distance of 3–4 cm using solvent 1: methylenechloride–methanol (98:2, v/v.) The plate was dried very carefully. The plate was then turned over 90° and run in the second direction using solvent 2: methylenechloride–propionic acid (98:2, v/v). As an alternative a '4×4' developing mode can be used. In this mode four samples were developed in two dimensions on one HPTLC plate [13]. After drying, fluorescence was induced by spraying or dipping with the alkaline cysteine solution (spray solution I+II). The identity of the spots followed from R_F comparison with derivatised standard solutions.

Derivatised standard solutions: 0.1 ml stock solution (=20 μg) was mixed with 5 ml buffer pH=8. After derivatisation and extraction the ether volume was reduced to 500 μl yielding a 40 ng/μl solution. One μl of these solutions was applied on the HPTLC plate as reference.

2.7. Multiple mass spectrometric confirmation

To shorten the length of the acronym MS–MS–MS . . . which is multiple stage mass spectrometry, it is preferred to use MS^n. In the first stage the pseudomolecular ion (MH^+) is fragmented. The second stage is a further fragmentation of the most intense fragment ion. These steps can be repeated until the fragments of the analyte fall apart.

2.7.1. Infusion

Infusion into the mass spectrometer was performed in two ways: in laboratory 1 (Ghent) the incoming flow of sample (5–10 $\mu l\,min^{-1}$) was directly introduced into the mass spectrometer. In order to exclude contamination the spray shield, heated capillary and infusion line were thoroughly cleaned with methanol before starting the infusion and acquisition. Eluent, 1% acetic acid–methanol (40:60, v/v) was infused into the mass spectrometer through the infusion line and checked for the presence of known derivative parent and daughter ions, to assure that no contamination or residues of previous standards or positive samples was present. If this check-up proved to be negative a new sample was infused. It should be noted that the spectrum of the analyte was not changed if another eluent mixture was used.

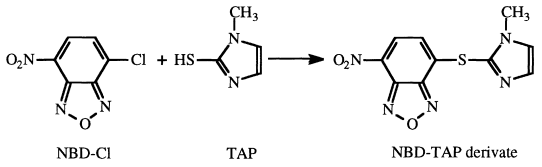


Fig. 3. Reaction between tapazole (M_r=114.2) and NBD-Cl (M_r= 199.5).

In laboratory 2 and 3 (Bilthoven) a laboratory-designed nanoelectrospray technique based on the work of Mann and Wilm was used [14,15]. In this technique a sample consumption of 10–20 nl min^{-1} from disposable capillaries was used and cross-contamination was impossible. With both techniques analogous results were obtained.

2.7.2. Tuning

The molecular mass of the derivatised analytes can be derived from the reaction of the thyreostats with NBD-Cl (Fig. 3). The MTU-NBD derivative was used to optimise the instrument. This component has a molecular mass approximately in the middle of the range of molecular masses of the analytes studied. A concentration (40 ng μl^{-1}) which produced a fairly distinguished pseudo molecular ion (MH^+) was directly infused into the mass spectrometer and the different MS parameters (capillary voltage, tube lens offset, ESI voltage . . .) were optimised and saved in a tune file. This tune file was used during the further investigation. In theory a tune file should be made for each compound individually to obtain the optimal conditions. In practice there was only a slight difference in parameter settings for compounds with a small molecular weight difference. This difference does not affect the sensitivity of the infusion.

Electrospray is a soft ionisation technique that produces MH^+ ions. Since this ion was the most abundant one, it was used for further fragmentation. As a general rule, multiple mass spectrometry was performed on the most abundant daughter ion, this to ensure detection of low concentration levels. During one acquisition MS^1 up to MS^3 or MS^4 was acquired. The relative value of the collision energy was chosen in relation to the maximal production of fragment ions. This value was comparable for related compounds. The scan range of the different fragmentation levels was noted in Table 2. For the different analytes the following rule was applicable:

MS^1 scan range: 250–350 relative molecular mass (this range contains all pseudo molecular ions of the derivatives of thyreostats studied in this application).

MS^n scan range: 150–(m/z parent ion + 5) relative molecular mass (none of the fragments have a molecular mass higher than its parent ion).

3. Results and discussion

3.1. Clean-up of thyreostatic drugs on mercury affinity columns

The binding characteristics of DBMF with Dowex 1 resins of various cross-linkages were tested and described previously [2]. Dowex 1-X2 was selected for clean-up of thyreostatic drugs. This resin binds 25 mg DBMF ml^{-1} resin, equivalent to 6.7 mg (33 μM) Hg^{2+} ml^{-1} wet resin.

Elution with 5 ml of 0.5 ml M NaCl solution (0.1 M HCl, pH 1) yields most of the thyreostatic drugs studied (TU, MTU, PTU, DMTU) at 80%. On the contrary, lower recoveries were noted for PhTU. The interaction of the phenyl group with the polystyrene matrix of the resin may explain the strong adsorption of PhTU. The elution yield of TAP was also lower (ca 60%).

Table 1
Most abundant ions for thyreostatic drugs

Thyreostat	MS^1	MS^2	MS^3
TAP	278, 267, 264	248, 232, 218, 202	217, 215, 202
TU	292, 291, 282, 235	262, 246, 245, 229, 216	202, 201, 177
MTU	306, 283, 282	260, 259, 244, 243, 230	203, 202, 201, 177
DMTU	320, 296, 288, 282	274, 273, 258, 257, 244	229, 203, 177
PTU	334	288, 271, 258	254, 243, 202, 177
MBI	314, 299, 279, 267	283, 269, 268, 254, 238	267, 253, 251, 238

3.2. Multiple mass spectrometry of standards of thyreostats

According to the law, a so-called 'positive' result of one sample, obtained in one laboratory can be challenged by the owner of the animal. Also at export/import, discussions between different countries about the reliability of the results of residue analysis may arise. The European Union Community Reference laboratory has to evaluate the results of the National Reference Laboratories and is often asked to confirm the results obtained in some countries in the Union.

In order to improve the qualitative support (to be really sure) of the analysis, the HPTLC method was coupled to a GC–MS method by scraping off the 'suspect spot' and after derivatisation injecting it into a GC–MS [3]. However, although the method works well at higher concentrations (spot>20 ng) the results were variable in the low concentration range. Moreover, the time needed for scraping off the spot, derivatising and injecting it again in a GC–MS was considerable. Therefore, we tried to improve both analytical accuracy and sample throughput by using MS^n.

The derivatised standard solutions of the thyreostatic drugs (40 ng μl^{-1}) were infused into the mass spectrometer working in the MS^n mode. Surprisingly, stable and undisturbed MS^1 spectra were obtained. Before, it was feared that the excess of NBD-Cl could disturb the ions of the derivative but no interference was observed. The basepeak of the spectra was the pseudo molecular ion (MH^+) corresponding with the expected mass from the reaction between the drug and NBD-Cl. An example of the reaction for TAP is given in Fig. 3.

Also the MS^2 and MS^3 spectra, taken on the most abundant ion gave typical ions. In Table 1 the most abundant ions for the thyreostatic drugs studied are given. In Fig. 4(A, B, C) the MS_n spectra for tapazole are presented.

Table 2
Scan ranges for thyreostatic drugs

	MS^2 scan range	MS^3 scan range
TAP	150–285	150–235
TU	150–295	150–235
MTU	150–310	150–245
DMTU	150–325	150–260
PTU	150–350	150–280
MBI	150–320	150–270

The nature of the interfering peak 304 in the MS-full scan spectrum of the tapazole derivative was unknown. The peak was not present in the infusate nor in the derivatising reagent. The presence of this peak has no influence on the further fragmentation of the pseudo-molecular ion (MH^+).

The formation of the fragment ions of TAP–NBD and TU–NBD is represented in Figs. 5 and 6. This is just a simple presentation. The breaking of the bond is given by dots. This should not be misinterpreted as a radical. Further justification of the fragmentation will be presented in the future. The analytes of which the most abundant daughter ion was formed through loss of NO_2 (−46) do not contain a hydroxyl group in their original structure (TAP). Thyreostats related to thiouracil form stable intense daughter ions through the loss of NO_2 and OH (−63).

As can be seen in the Table 1 and in Fig. 4 all thyreostatic drugs give at least eight diagnostic ions for identification. As well the parent as the daughter and granddaughter ions are important. Of course, the table and the figure do not give the exact m/z value of the ions: depending upon the calibration of the mass spectrometer these values, given in one digit after the period can vary a little within acceptable limits. Also depending on the collision energy of the parent ion the relative abundance of the fragment ions can vary.

3.3. Multiple mass spectrometric confirmation of samples

After being successful with standards, some samples out of regulatory control were infused.

In routine analysis samples are screened with HPTLC. The MS^n step is a confirmation of suspect samples. The energy applied on the ions of interest to obtain a fragment ion spectrum was the same for standards and for 'real' samples. Both spectra were compared and if there was a match, the presence of the analyte was indicated. Concerning endogenous material present in 'real' samples, it is possible that matrix background contains the same parent ions (MH^+) as the analytes of interest. Even if these interfering ions mask the MS-full scan (single MS)

presence of the analyte, the second stage and further stages of MS will eliminate the doubt. The MH^+ ion is isolated and fragmentation occurs. If the MH^+ ion of an endogenous compound was the same as MH^+ of an analyte the fragmentation spectrum will be completely different. If second stage MS (MS–MS) is not convincing a fragment ion can be isolated (the most intense daughter ion of the standard spectrum) to fragment it further (MS–MS–MS) and compare again with the spectrum of the standard. Identification was performed, following the same recipe as for standard. There will be no endogenous compound having the same MS–MS, MS–MS–MS, . . . , MS^n spectrum as one of the thyreostatic drugs detected in this application.

To the remainder of the extract (50 μl from which

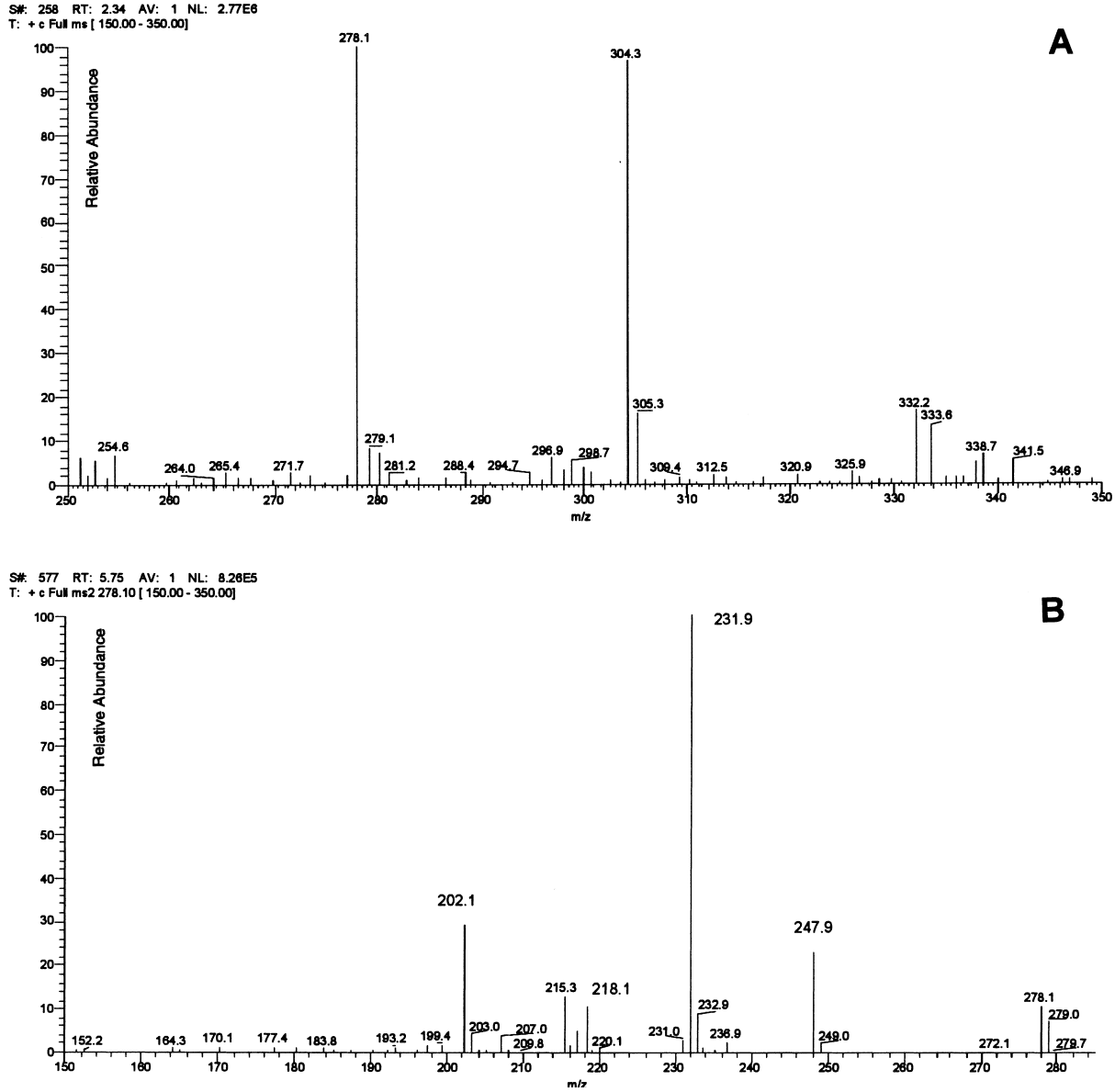


Fig. 4. MS (A), MS^2 (B) and MS^3 (C) spectra of the tapazole-NBD derivate by direct infusion into the mass spectrometer (MH^+=278.1).

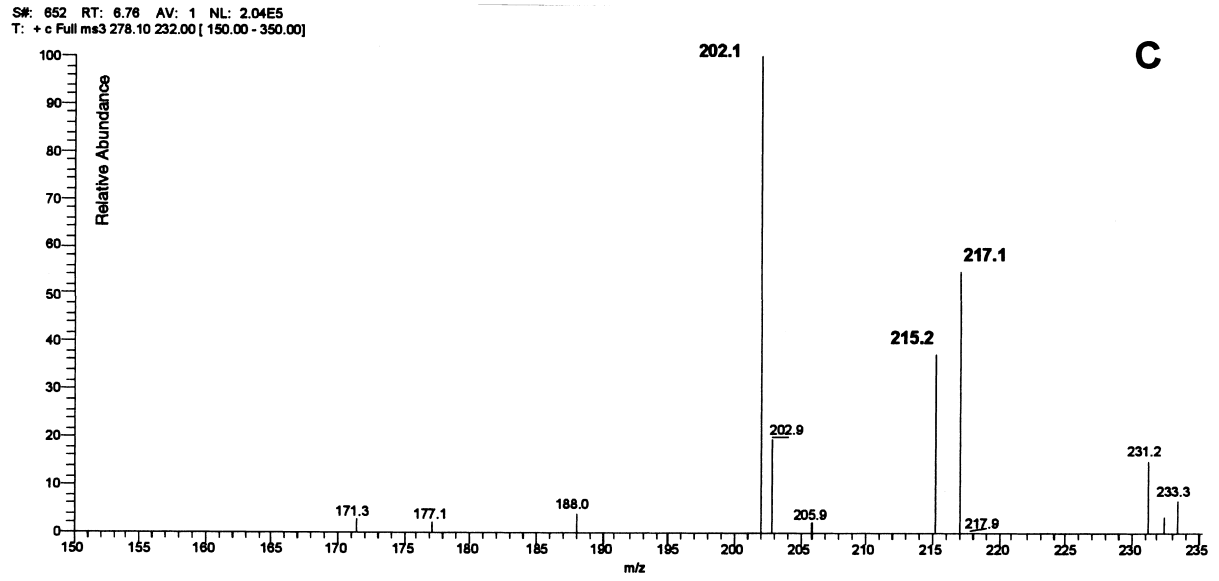


Fig. 4. (*continued*)

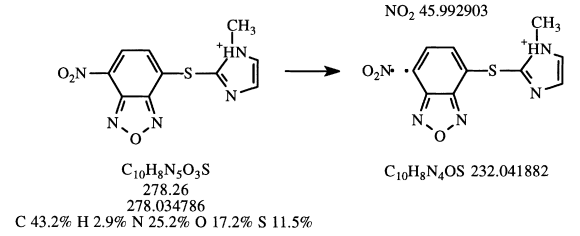


Fig. 5. Fragmentation of the parent tapazole-NBD derivative yielding the daughter ion 232.

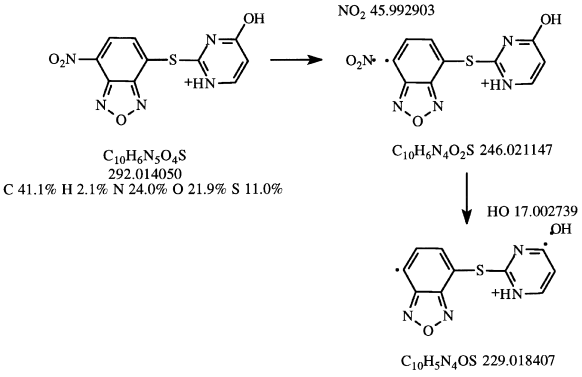


Fig. 6. Fragmentation of the parent thiouracil-NBD derivative yielding the daughter ions 246 and 229.

10 μl was spotted) 250 μl of diethyl ether was added and directly infused into the LCQ. An example of the results of a thyroid sample out of routine analysis (K970315B) is given in Fig. 7.

As can be seen the MS^1 spectrum taken on the extract of the thyroid sample was dominated by the base peak 282.5. In each spectrum taken on thyroid extracts this peak was detected (until now). This peak divides the interesting mass scale into two areas. The molecular masses of the most derivatised thyreostats were situated above 282.5 (see Table 1). So, it was advisable to start the MS^1 mass range with a mass higher than 282.5 (e.g., 285). Unfortunately, the molecular mass of the TAP–NBD derivative, which is important, was lower than the base peak found in the thyroid extracts.

In all the extracts, taken from routine the presence of the internal standard (DMTU) added was detected by TLC. In the MS^1 spectrum a small peak with a value of 320, corresponding with the molecular mass of DMTU was observed. When MS^2 and MS^3 was performed on this small 320 peak as a parent ion the correct $MS^{2,3}$ spectra of DMTU resulted (Fig. 7A, B, C).

The EU Community Reference Laboratory received thyroids in which tapazole was detected with

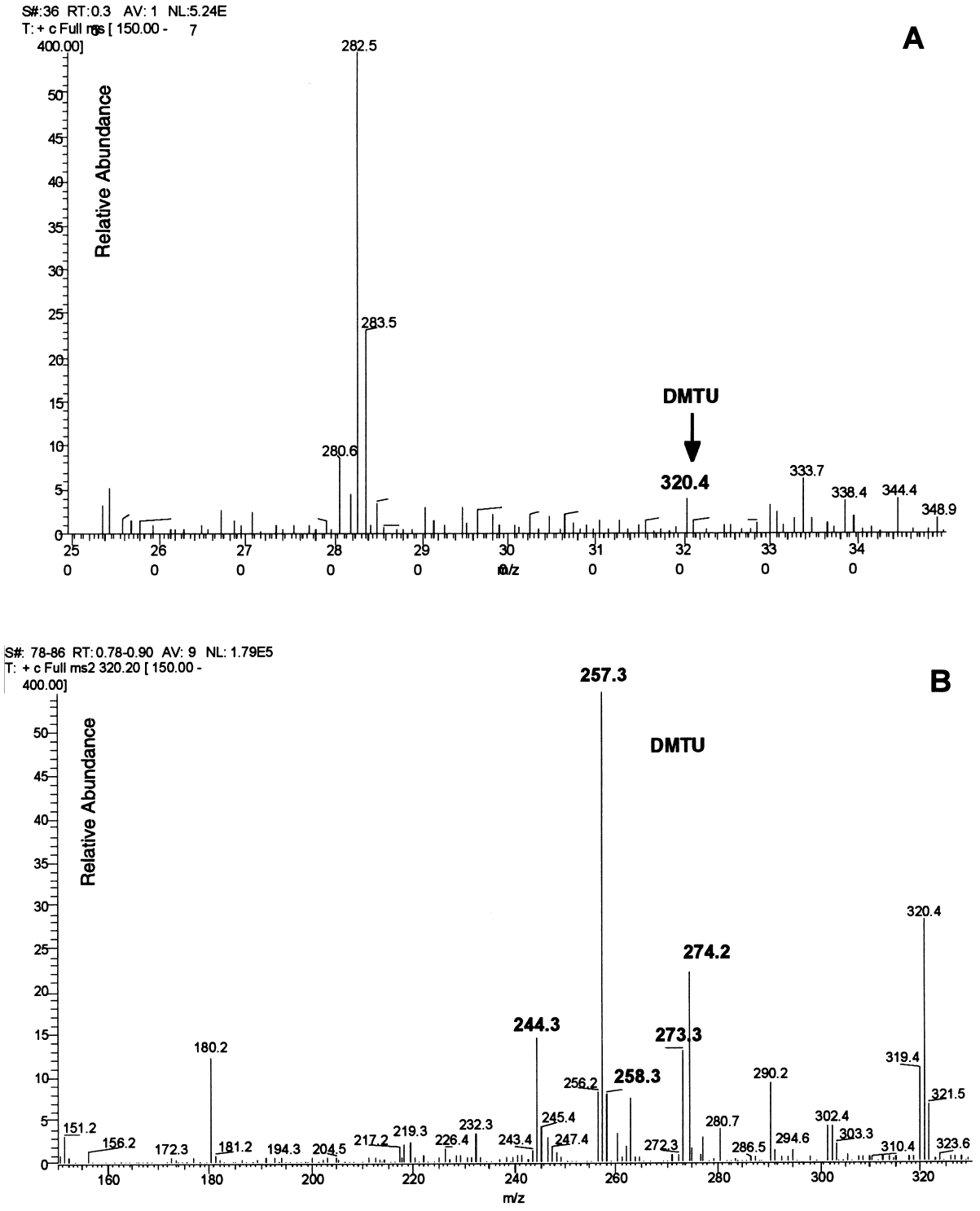


Fig. 7. MS (A), MS^2 (B) and MS^3 (C) spectra of thyroid sample K970315B.

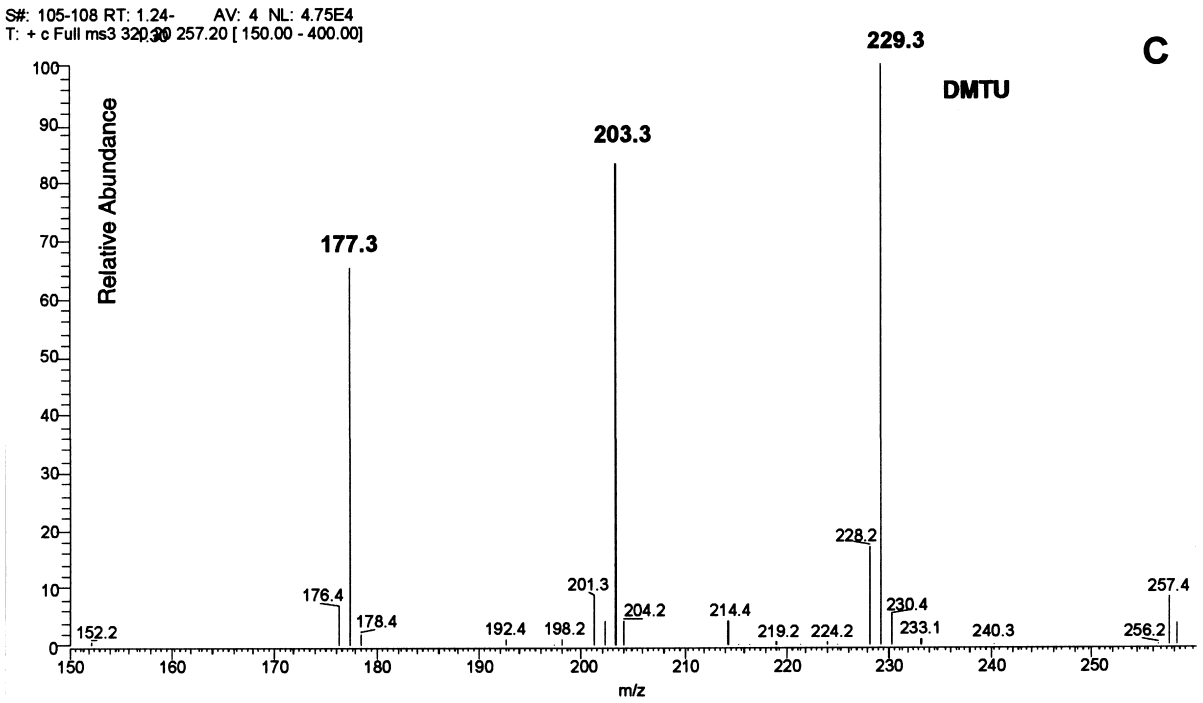


Fig. 7. (*continued*)

HPTLC in one of the member states. In Fig. 6 the MS^n spectra of such a thyroid sample are given.

As can be seen in Fig. 8 the correct five daughters of ions of peak 278 in the MS^2 spectrum and the correct three granddaughters of peak 278 (through ion 232) on the MS^3 spectrum were present. From the combination of these spectra and the presence of a fluorescent spot at the two correct R_F values in two-dimentional HPTLC the presence of tapazole was considered to be proven.

3.4. Quality criteria for multiple mass spectrometry

The use of multiple mass spectrometry in confirmatory analysis of residues brings up the problem of the quality criteria to be used. In Commission Decision 93/256 on quality criteria [16] four diagnostic ions were needed at the correct retention time showing the correct relative abundances using classical GC–MS in the selected ion monitoring (SIM) mode. The ratio of the abundances of these ions must correspond with the standard within 10% (electron impact, E1) or 20% (chemical ionization, CI). It will not be easy to translate or adapt these criteria to MS^n. At first, no retention time is available. It is possible to make a HPLC separation but it will be more time consuming. In performing a direct infusion or in doing an LC separation, coeluting peaks can in both cases be separated mass spectrometricly in MS–MS or MS^n by filtering out of the full scan spectrum only those ions of interest.

Moreover, the ratios of the abundances of the ions in MS^n will not be so stable as in GC–MS-SIM systems. In derivatised analytes, as the TS–NBD derivatives are, the nature of the fragment ions will also be important. It should be taken into account if the fragment ions were derived from fragmentation of the analyte itself (e.g. TAP) or from the derivatisation agent (e.g., NBD) (Fig. 5).

From a legal point of view, since this is a confirmation of suspect samples screened with TLC, the R_F values of the analyte correspond with the R_F

value typical of the standard analyte. The results fulfil the criteria of 93/256/EEC. The criterium of an allowed margin of 20% in CI is not applicable to infusion MS. The ionisation mode was electrospray and the margin in the criterium is 'preferably' +10% for EI or +20% for CI. For this application there are no criteria for MS–MS or multiple MS. So combining the TLC and the confirmatory power of multiple MS, from a legal point of view, the results of positive samples are sufficiently supported. However other combinations of quality criteria will have to be worked out [17–19].

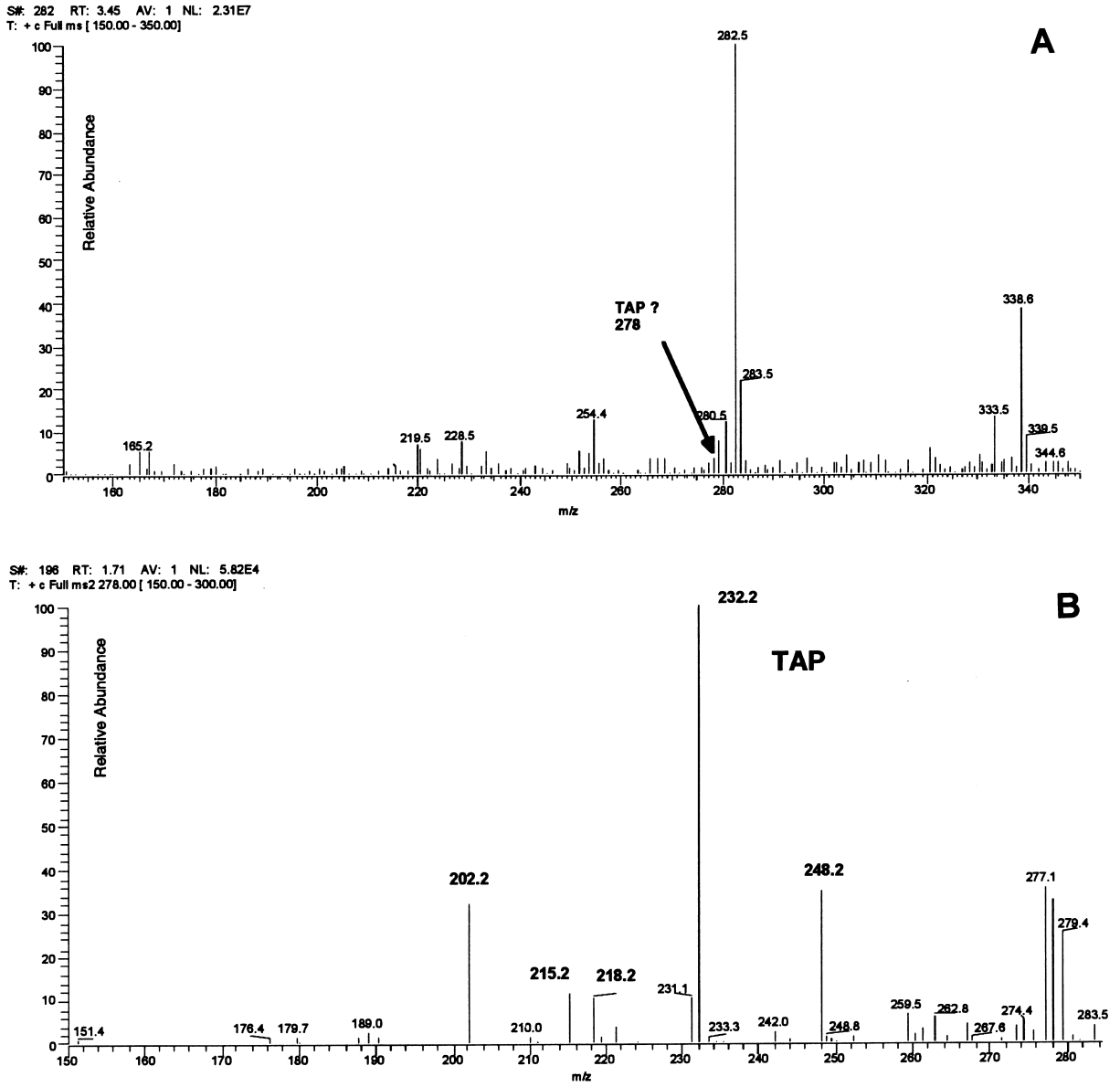


Fig. 8. MS (A), MS^2 (B) and MS^3 (C) spectra of thyroid sample R970032C showing the presence of tapazole on the daughter and granddaughter ions of peak 278.

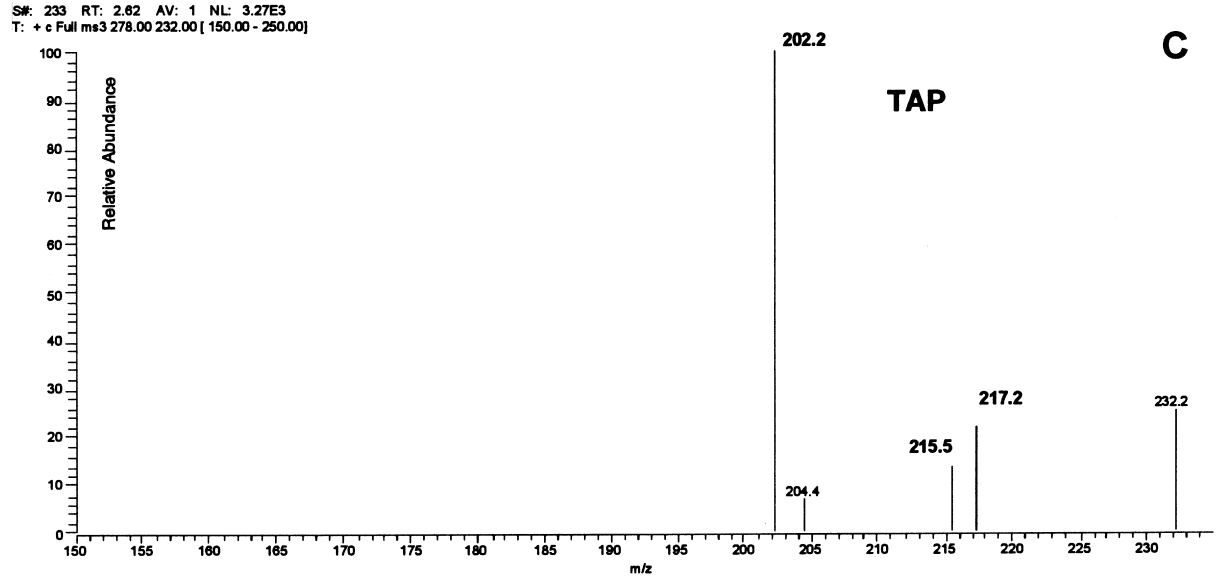


Fig. 8. (*continued*)

4. Conclusion

For qualitative analysis of thyroid tissues, as needed in standard EU routine control, the rapid clean-up procedure, coupled with the specific fluorescence detection after HPTLC remains important for screening of the possible abuse of thyreostats. Using infusion is a fast way of identifying analytes in suspect samples. Chromatographic separation takes more time developing the method but can also be done. In doing so by working in MS–MS or a higher state of MS, the analysis is focused on target compounds. In viewing the spectrum of the sample in a continuous way a lot of very interesting information is gained about unknown compounds. Next to MTU, TU and PTU the method could also be used for routine control of TAP but with a higher detection limit (100 ppb instead of 25 ppb) using DMTU as internal standard. From data of animal experiments it is proved that if thyreostats are abused residues are detected in tissues and excreta during a long withdrawal period.

The coupling of the HPTLC method to a MS_n instead of a GC–MS confirmation improves both the qualitative support of the analysis and the sample throughput. The identity of the residue follows from two R_F values on a HPTLC plate, a characteristic fluorescence induction, coupled to three related mass spectra containing (at least) eight characteristic ions.

Acknowledgements

This work was supported by a grant of the Belgian Ministry of Public Health (Institute of Veterinary Inspection IVK-IEV). We thank W. De Rycke for expert technical assistance.

References

[1] H.F. De Brabander, R. Verbeke, J. Chromatogr. 108 (1975) 141–151.
[2] H.F. De Brabander, The determination of thyreostatic drugs in biological material, Thesis, University of Ghent, Ghent, 1986.
[3] H.F. De Brabander, P. Batjoens, J. Van Hoof, J. Planar Chromatogr. 5 (1992) 124–130.
[4] P. Batjoens, H.F. De Brabander, K. De Wasch, J. Chromatogr. A 750 (1996) 127–132.

[5] R. Schilt, J.M. Weseman, H. Hooijerink, H.J. Korbee, W.A. Traag, M.J. Van Steenbergen, W. Haasnoot, J. Chromatogr 489 (1989) 127–138.
[6] J. Buhlert, Dtsch Lebensm.-Rundsch. 82 (1986) 146–148.
[7] B. Le Bizec, F. Monteau, D. Maume, M.-P. Montrade, C. Gade, F. André, Anal. Chim. Acta 340 (1997) 201–208.
[8] A. Poncelet, DEA Physico-Chimie des Bio-Produits, Nantes, 1995, p. 40.
[9] N. Le Marrec, Thèse d'Université, Nantes, 1995, p. 184.
[10] W.J. Blanchflower, P.J. Hughes, A. Cannavan, M.A. McCoy, D.J. Kennedy, Analyst 122 (1997) 967–972.
[11] R. Verbeke, J. Chromatogr. 177 (1979) 69–84.
[12] H.F. De Brabander, G. Rasschaert, W. Vanneste, J. Planar Chromatogr. 2 (1989) 484–485.
[13] H.F. De Brabander, F. Smets, G. Pottie, J. Planar Chromatogr. 1 (1988) 33–38.
[14] M.S. Wilm, M. Mann, Int. J. Mass Spectrom. Ion Proc. 136 (1994) 167–180.
[15] M.S. Wilm, M. Mann, Anal. Chem. 68 (1996) 1–8.
[16] EEC Directive 93/256, European Union, Brussels, 1993.
[17] H.F. De Brabander, G. Pottie, D. Courtheyn, F. Smets, in: Proceedings of Euroresidue III, Veldhoven 5th–8th May 1996, pp. 283–287.
[18] D. Courtheyn, H.F. De Brabander, P. Batjoens, J. Vercammen, K. De Wasch, Proceedings of Euroresidue III, Veldhoven 5th–8th May 1996, pp. 75–85.
[19] R. Stephany, J. Chromatogr 489 (1989) 3–9.