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Validation of a method for the detection and confirmation of nitroimidazoles and corresponding hydroxy metabolites in turkey and swine muscle by means of gas chromatography–negative ion chemical ionization mass spectrometry

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Abstract

The results of a validation study of a GC–NCI–MS method for the quantitative determination of 5-nitroimidazoles {1,2-Dimethyl-5-nitroimidazole (dimetridazole, DMZ), 1-methyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole (ronidazole, RNZ), 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole, MNZ) and 2-isopropyl-1-methyl-5-nitroimidazole (ipronidazole, IPZ)} including the hydroxy metabolites of these agents {2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI), 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (MNZOH), and 1-methyl-2-(2′-hydroxyisopropyl)-5-nitroimidazole (IPZOH)} in turkey and swine muscle are presented. The validation was carried out according to the requirements of the draft for the revision of Commission Decision 93/256/EC, which is expected to be adopted by the European Commission in due course. The determination of the method's performance parameters revealed decision limits (CC_α) between 0.65 and 2.8 μg/kg for DMZ, RNZ/HMMNI, MNZ and MNZOH. Confirmatory analyses according to the requirements of the forthcoming EC decision are possible for all analytes except for IPZ and IPZOH where already the decision limits (CC_α) were higher (5.2 μg/kg) than for the above-mentioned nitroimidazoles. The within-laboratory reproducibility and the mean recovery were in an acceptable range for all analytes. Published by Elsevier Science B.V.

Keywords: Nitroimidazoles

1. Introduction

5-nitroimidazoles had been widely used in veterinary medicine for the treatment and prophylaxis of histomoniasis in poultry and haemorrhagic enteritis in pigs before they were banned from use in food producing animals [1] because carcinogenic and mutagenic properties could not be excluded [2].

Because of their high misuse potential 5-nitroimidazoles are normally included in the monitoring programmes for residues of veterinary drugs. To carry out the requested controls, reliable and sensitive methods are necessary, which are able to detect the substances in low concentrations and to un-

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ambiguously confirm the presence of the analytes in suspected samples.

In literature several analytical methods for the determination of nitroimidazoles in matrices using high-performance liquid chromatography (HPLC–MS) [3–7] and also HPLC–MS–MS [8] are described. In contrast, only a small number of methods using gas chromatography (GC) [9,10], GC–MS and GC–MS–MS [11] are reported. Consequently, there is still a lack of routinely applicable multi-residue methods using this technique. GC offers a high separation capacity and, when coupled to mass spectrometry (MS), achieves a sensitivity and a high specificity which so far have not been equalled in LC–MS. The HPLC–MS–MS method [8] delivers results in the same order of magnitude, but requires a much more demanding and cost-intensive analytical equipment. Thus the GC–MS is offering good prospects for the confirmation of the analytes by means of a relatively cost-saving technique. Especially the application of the negative chemical ionisation–mass spectrometry (NCI–MS) technique for the detection of this substance group seemed to be very promising, since these substances facilitate the addition of electrons thanks to their nitro group. Additionally, signals of interfering substances are suppressed due to the selective ionisation [11,12].

2. Experimental

The method comprises the homogenisation of the sample, an enzymatic hydrolysis, a solid-phase extraction using kieselguhr cartridges, a derivatisation and a GC–MS measurement.

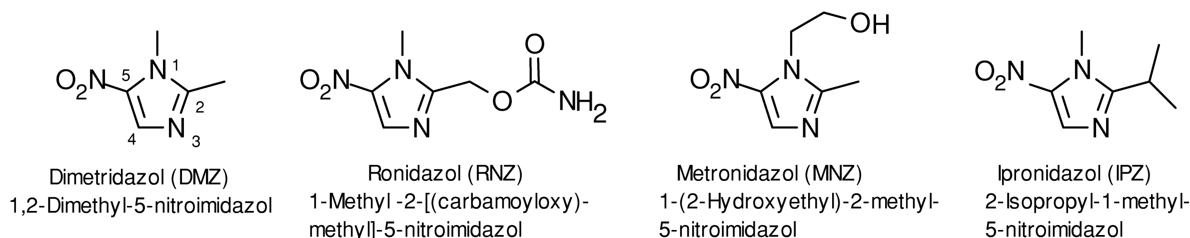
2.1. Chemicals and reagents

The organic solvents were purchased from Merck (Darmstadt, Germany) in suprasolv quality. The analytical standards RNZ, DMZ and MNZ were purchased from Sigma (Deisenhofen, Germany), HMMNI from Merck (Darmstadt, Germany), MNZOH from Rhône-Poulenc Rorer Ltd. (Dagenham Essex, UK), IPZ from aniMedica (Horb am Neckar, Germany), IPZOH from FDA (Washington DC, USA), 1-(3-hydroxypropyl)-2-methyl-5-nitroimidazole (ternidazole, TNZ), D_3-IPZ, D_3-IPZOH and D_3-HMMNI from Witega (Berlin, Germany) and D_3-RNZ and D_3-DMZ from RIVM (Bilthoven, The Netherlands). A sodium chloride–potassium bihydrogen phosphate ($NaCl–KH_2PO_4$) buffer solution is prepared from 5.84 g of NaCl and 13.61 g of KH_2PO_4 (both purchased from Merck, p.a. quality) in 900 ml of water, adjusted to pH 3 and filled up to 1000 ml with water. For the enzymatic hydrolysis Protease Type XVIII, Sigma (Deisenhofen, Germany) was used, the solid-phase extraction was performed with Extrelut NT20 (Merck) and the samples were derivatised with N,O-bis-(trimethylsilyl)acetamid (BSA, derivatisation grade), Machery-Nagel (Düren, Germany) (Fig. 1).

2.2. Sample preparation

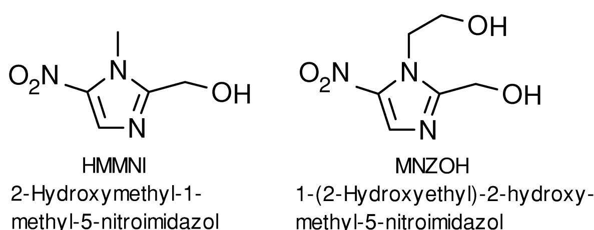
5 g portions of muscle samples (fresh or frozen material) were cut into small pieces and transferred into a stomacher bag. Then the analytes and the internal standards, 6 ml of buffer solution, and 1 ml of protease solution were added and the mixture is homogenised for 4 min in a stomacher (Seward, London, UK). The samples, which should have a pH value of 3, were then hydrolysed at 37°C overnight. Subsequently they were centrifuged at 2600 *g* and at 4°C for 15 min. The upper layer was decanted and 8 ml of buffer solution were added again to the remainder, which was then suspended. Following centrifugation, the upper layer was also decanted and the aqueous layers were combined, adding up to approximately 20 ml of buffer solution. The aqueous solution was defatted by a two-fold careful extraction with 10 ml hexane each. The remaining aqueous phase was adjusted to pH 6 and filled up with buffer solution to 18 ml. The solution was applied to an extrelute cartridge. After 20 min equilibration time, 20 ml of ethyl acetate (EtAc)–tert.-butyl-methylether (tBME), 1:1 v/v, were applied. After another 15 min equilibration time, 2×20 ml of EtAc–tBME solution were added to elute the analytes. The combined eluates were concentrated to 0.5 ml in a TurboVap evaporator (Zymark, Idstein, Germany). The concentrate was transferred into a derivatisation tube, evaporated to dryness under a weak nitrogen stream and derivatised with a solution of 50 μl of BSA–50 μl of *i*-octane for 60 min at 50°C to produce trimethylsilyl-derivatives (TMS-derivatives). The resulting mixture was directly injected into the GC–MS system.

Analytes



Metabolites

int. Standard



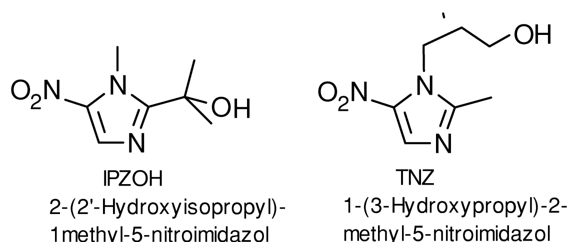


Fig. 1. Analytes and internal standards employed in the validation study. (Deuterated internal standards having a CD_3-group in the 1-position).

2.3. GC–MS instrumentation

2.3.1. GC columns

For the validation experiments a 30 m×0.25 mm I.D. column with 0.25 μm of methyl-5%-phenylsilicone (ZB 5, Phenomenex, Aschaffenburg, Germany) was used as the standard column. In the course of the method development, two further columns were tested for nitroimidazoles with respect to their separation behaviour: A 30 m×0.25 mm I.D. column with 0.25 μm of methylsilicone (ZB 1, Phenomenex, Aschaffenburg, Germany) and a 30 m×0.25 mm I.D. column with 0.25 μm of methyl-35%-phenylsilicone (ZB 35, Phenomenex, Aschaffenburg, Germany).

Each of the columns was used with a pre-column: 5 m×0.32 mm I.D., deactivated (Hewlett-Packard).

2.3.2. NCI measurements

A HP 5973N mass spectrometer coupled with a GC (HP 6890 plus) and equipped with an automatic sampler (HP 7683) was used for the measurements. One μl of the sample was injected in splitless mode (1.5 min splitless time). The flow-rate was 1.0 ml/min, the injection block temperature 285°C. The separation was performed using an oven temperature programme: Initial temp. 85°C; 1.5 min, 15°C/min to 100°C; 5°C/min to 140°C; 10°C/min to 190°C; 30°C/min to 290°C (5 min). The interface was held at 285°C. The mass spectrometer was operated in the negative chemical ionisation mode (NCI), using methane as CI gas at an ion source pressure of $2.2–2.4\times10^{-4}$ Torr and a temperature of 160°C. The detection was performed in the selected ion monitoring mode (SIM), each of the analytes (including the corresponding internal standard) in a separate time window.

3. Results and discussion

3.1. Sample preparation

The pre-treatment of the muscle material, i.e. the homogenisation by stomaching as well as hydrolysis with protease overnight, has proved worthwhile,

since the following sample preparation becomes more reproducible and less interferences occur. An emulsification or any interferences of the extraction through the obstruction of the extrelute cartridges did not take place when proceeding like that. However, the first experiments with incurred material suggest that the hydrolysis does not lead to significant differences in the measurable analyte content [13]. As the analytes degrade quickly at room temperature in the matrix, a fast treatment of the sample up until the beginning of the hydrolysis is necessary [14]. In contrast the degradation of the analytes caused by daylight seems to play a minor role, since the loss of analytes in a standard solution stored in daylight at room temperature compared to a solution stored in the dark at −20°C is less than 10 to 15% within a week (Table 1).

The extrelute material behaves in a reproducible manner and guarantees the retention of water during the extraction with EtAc–tBME for a maximum volume of 18 ml of aqueous solution.

The concentration of the extracts by means of TurboVap allows a quick, reproducible and gentle solvent reduction to a volume of 0.5 ml, which is then evaporated to dryness just before the derivatisation. This proceeding produces absolute analyte yields of 15–25% for IPZ and IPZOH, of 65–75% for RNZ, of 45–60% for DMZ and of 50–60% for MNZ and MNZOH. The yields were calculated from the ratio of the peak areas of the internal standard in the sample to the mean peak area of the internal standard of the corresponding standard calibration. The small yields for IPZ and IPZOH are mainly caused by the defatting step, during which large amounts of analyte are, also at pH 2 or pH 3, extracted by the *n*-hexane. If more polar solvents as e.g. tBME are used for defatting, these two substances might even be extracted nearly entirely. The derivatisation reaction of MNZ, MNZOH, IPZOH and HMMNI to the trimethylsilyl compounds with BSA is rather quick and reproducible and does not modify DMZ and IPZ. However, the carbamoyloxymethyl group of RNZ is broken up and the same trimethylsilylether is produced which also results from the transformation of HMNNI. For this reason, a distinction between RNZ and the common metabolite of DMZ and RNZ, HMMNI (Fig. 2), cannot be made with this method.

Yet, since this derivative indicates, in any case, the presence of a banned substance, the application of this method for control purposes is justified.

3.2. Detection of the analytes

For the separation of the samples different GC-separation phases were examined. The deuterated standards could never be separated completely. On a pure methylsilicon phase the separation of MNZ and IPZOH was not possible. The best separation results were achieved with a ZB5-column, which was then used for the validation experiments. The NCI spectra were recorded under the conditions mentioned in the experimental section. The scan range was 40 to 400

Table 1
Degradation of nitroimidazoles in a methanolic standard solution in daylight

	Measured start concentration [μg/l]	Storage conditions	Storage time		
			1 week	2 weeks	4 weeks
DMZ	0.99±0.04	−20°C	0.91	0.95	0.95
		r.t./daylight[a]	0.86	0.82	0.67
RNZ	1.00±0.05	−20°C	0.96	0.98	0.96
		r.t./daylight[a]	0.90	0.87	0.75
IPZ	0.99±0.05	−20°C	0.94	0.94	0.96
		r.t./daylight[a]	0.87	0.88	0.66
MNZ	0.99±0.05	−20°C	0.95	0.92	0.93
		r.t./daylight[a]	0.88	0.76	0.65

[a] Storage in a clear glass bottle at a sunny window.

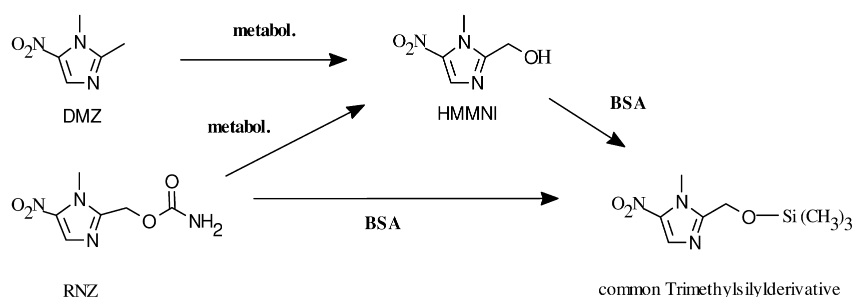


Fig. 2. Metabolisation and derivatisation of DMZ and RNZ.

amu. The characteristic ions are summarised in Table 2.

In general the non-derivatised members of this substance group and the members hydroxylated at the 1-alkyl group (and converted to TMS-ethers) provide the molecule ions as base ions (DMZ, IPZ, MNZ, TNZ). Those 5-nitroimidazoles which are hydroxy-substituted at the 2-alkyl group and converted to trimethylsilyl derivatives provide intensive ions on M-90. This is probably due to the loss of trimethylsilyl hydroxid {$(CH_3)_3SiOH$)} involving the alkyl group in the 1-position, as can be seen from the comparison to the fragments of the deuterated internal standard.

Fragments corresponding to M-16 and M-32 can be found with all the nitroimidazoles presented here in a more or less intensive degree. The ion corresponding to m/z 46 (NO_2^-), which is characteristic of the nitro-group, can also always be found, however only with MNZOH does it occur in intensities of >0.2% rel. intensity (normalised to the base ion).

With regard to the confirmatory analysis the use of NCI–MS as a detection method resulted in lower detection limits than those achievable with EI–MS and allowed the determination of more analytes in the concentration range investigated than with EI–MS–MS, respectively [11,13].

3.3. Validation

For the method validation, fortified blank material was utilised. Altogether, three different samples of swine muscle and three different samples of turkey muscle, each of a different origin and being ex-

Table 2
Selected ion monitoring (SIM)-masses, ions for the quantification and ion relations of the characteristic ions for the examined analytes (concentration range 0.5–6 μg/kg, see Table 3)

	Diagnostic ions (SIM ions)	Mean ion ratio	Confirmation possible?
DMZ	**141**, 142, 125, 109	100:7:7:7	4 ions
IS	D_3-DMZ, **144**		
RNZ	**139**, 230, 229, 197, [107]	100:7:47:18	4 ions
IS	D_3-RNZ, **141**		
MNZ	**243**, 244, 227, 211	100:16:4:4	4 ions
IS	TNZ, **257**		
MNZOH	**169**, 332, 331, [139], 46	100:17:72:3	4 ions
IS	TNZ, **257**		
IPZ	**169**, (170), 153, (137)	100:(20):5:(5)	2 ions[a]
IS	D_3-IPZ, **172**		
IPZOH	**167**, (257, 225),151,	100:(10:20):8	2 ions[a]
IS	D_3-IPZOH, **170**		

Bold figures: Ions for quantitation; in [] brackets: Ions which may be used for confirmation too; in () brackets: Ion traces strongly influenced by matrix effects.

[a] A confirmation is not possible due to matrix interferences of two ion traces.

amined in a fresh, slightly tainted as well as deep frozen state, were analysed. This sample material was fortified with the analytes and the internal standards, resulting in three analytical series with three concentration levels each and seven samples per concentration level (Figs. 3–5).

Moreover, one matrix calibration was carried out per series. These calibration curves consisted of equidistant measurement points with concentration levels of 0, 0.5, 1.0, 1.5, 2.0, 2.5 μg/kg for RNZ, and of 0, 1.5, 3.0, 4.5, 6.0, 7.5 μg/kg for DMZ, MNZ, MNZOH, IPZ and IPZOH. In order to prove the specificity, several blank samples and blank samples fortified with an internal standard only were additionally analysed in each series.

3.4. Repeatability, within-laboratory reproducibility and recovery (internal standard-corrected)

The samples were analysed by means of GC–NCI–MS in SIM and quantified on the basis of the relevant matrix calibration curve with the help of the internal standards. The internal standards employed for this purpose as well as the masses of the single ion traces can bee seen in Table 2.

The confirmation of the analytes was effected according to the draft revision of Commission Decision 93/256/EG [15][1] by using a fortified blank matrix. The repeatability and within-laboratory reproducibility were calculated by using single factorial analysis of variances (ANOVA) for each of the three concentration levels (Table 3).

3.5. Determination of CC_α and CC_β

According to the draft revision of Commission Decision 93/256/EC, analytical methods for banned substances are to be validated at zero-level. In order to reach the greatest possible harmonisation of the European residue control of pharmacologically active substances in food-producing animals, a Minimum Required Performance Level (MRPL) was introduced, at which these methods are to be validated. Since these MRPLs have not yet been established, an auxiliary parameter had to be used to carry out this validation study. For this purpose, in preliminary tests appropriate concentration levels were determined which should still safely allow a reproducible confirmation of the substance. The concentrations of the analytes in the three repeatability series studied were then chosen in such a way that the lowest concentration was within the range of the assumed minimal concentration that could still be confirmed, and the next levels were set at the 1.5- and 2-fold of that concentration (Table 4).

The matrix calibrations belonging to the different series were constructed in such a way that the corresponding concentration ranges were covered by at least 4 equidistant concentration levels plus the zero-level.

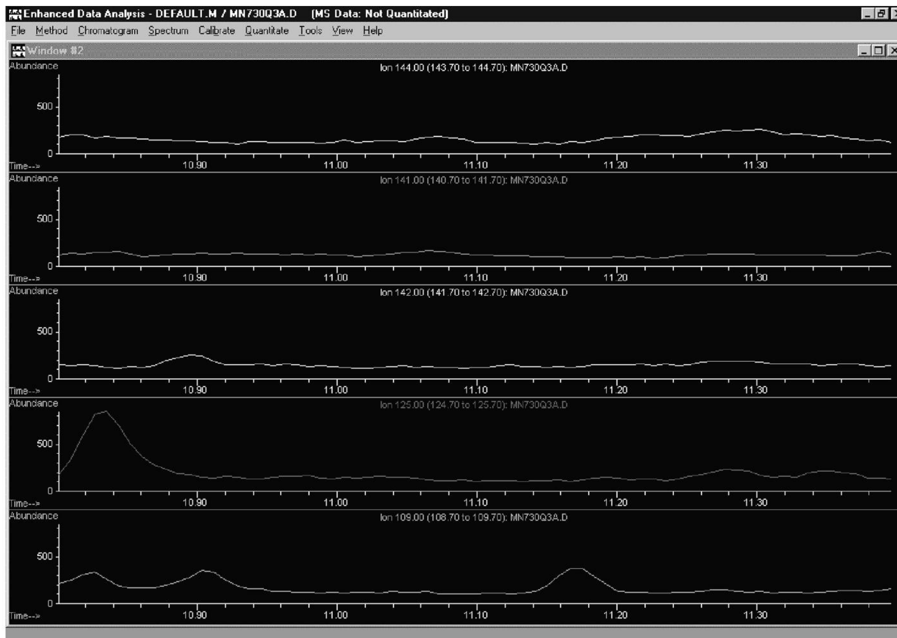
Three different options for the calculation of CC_α were chosen. For all of the options the same data set was used, but different approaches of calculation were applied. Table 4 shows the results of these calculations.

The aim of the present study was the validation according to the criteria of the draft revision of Commission Decision 93/256/EC [16]. The two proceedings described there will be presented as variants a and b. The calculation of CC_α according to variant a is performed mostly in agreement with the regulations of the draft revision for calibration curve proceedings, i.e. the determination of the *y*-intercept is effected by means of a linear extrapolation of the matrix calibration curve and the addition of the 2.33-fold within-laboratory standard deviation of the lowest concentration level[2]. Table 4 lists the results obtained from this calculation. It becomes apparent that this proceeding results in very low CC_αs, which are far below the lowest still identifiable fortification levels established in preliminary tests. Therefore, these CC_αs are not suitable to reflect the real proficiency for confirmatory purposes.

As a second variant (variant b), the blank matrix

[1] The criteria for a successful substance confirmation require constant relative retention times (deviations <±0.5%) and constant ion ratios for 4 characteristic ions (EI–MS and CI–MS). For this purpose, the sample to be confirmed is either compared to a standard or to a fortified blank matrix. The permitted tolerances depend on the relative intensities of the individual ions.

[2] The SANCO/1805/2000 requires the addition of the within-laboratory reproducibility standard deviation of the *y*-intercept. In the view of the authors the *y*-intercept has no standard deviation, therefore the one of the lowest concentration level was chosen.



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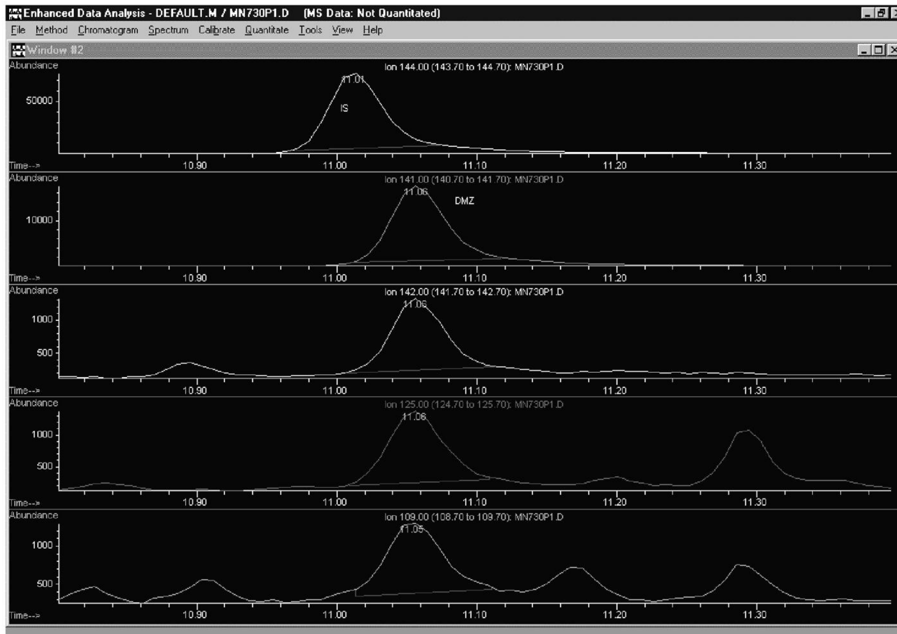
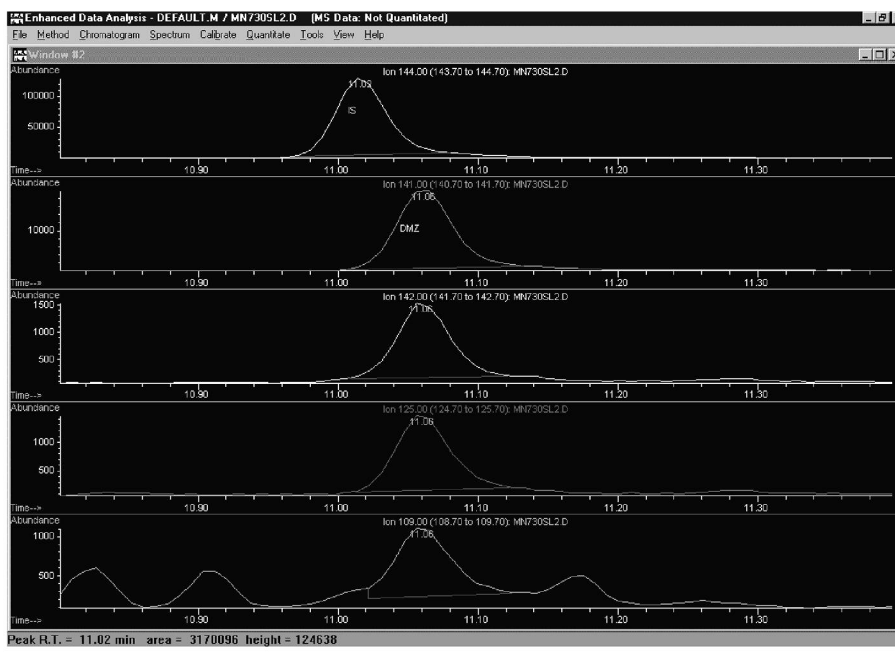


(b)

Fig. 3. (a) Matrix blank (turkey muscle) for DMZ; (b) 2 μg/kg DMZ in turkey muscle, internal standard at 10 μg/kg; (c) 1.5 μg/kg DMZ in a standard sample, internal standard at 10 μg/kg.



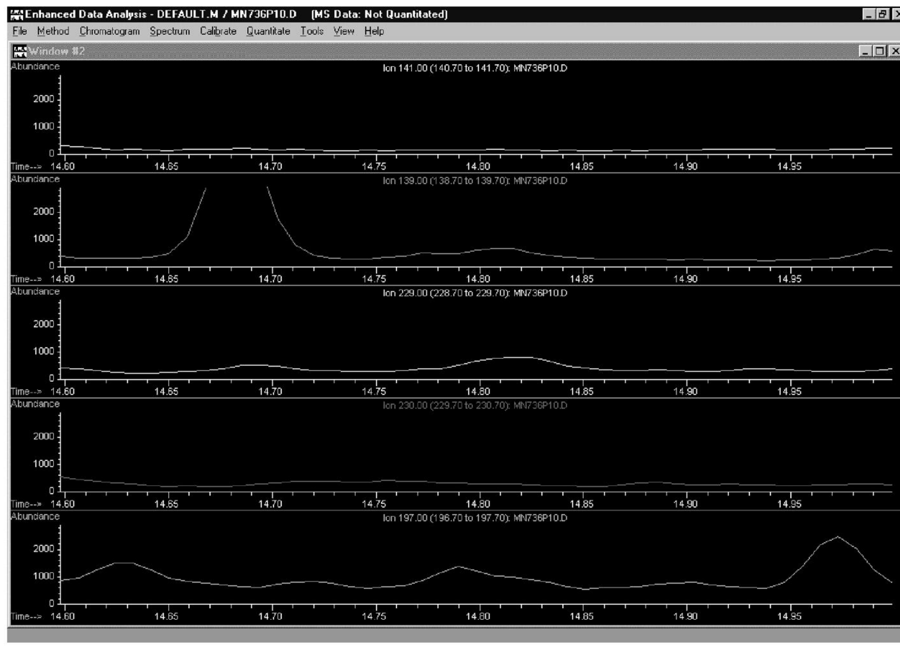
(c)

Fig. 3. (*continued*)

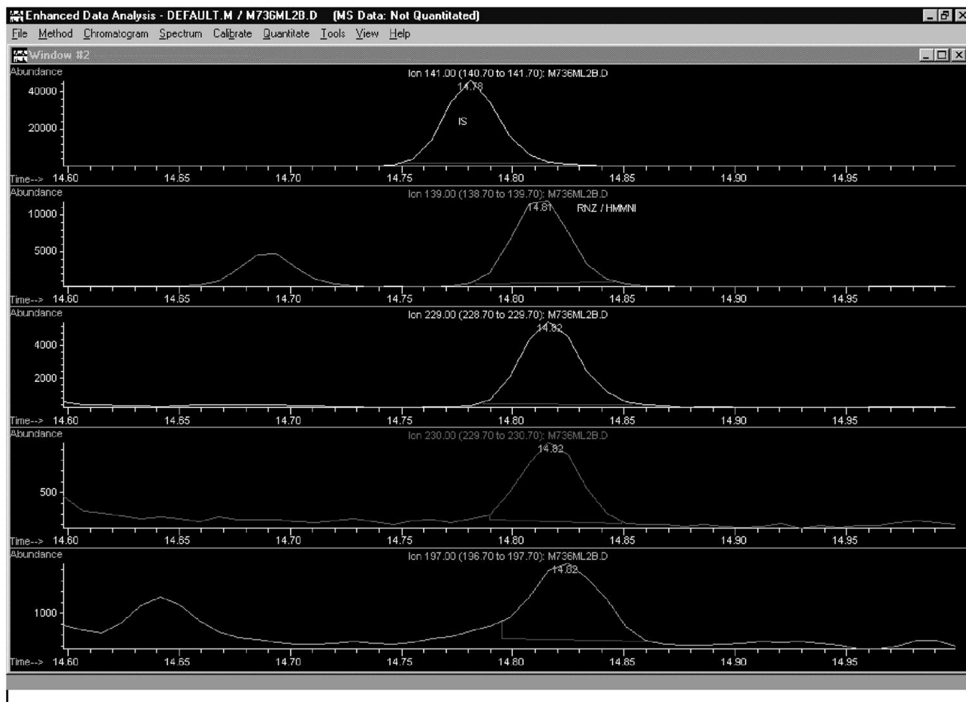
proceeding was applied, which according to the draft revision can also be employed in the framework of a classical validation (variant b, Table 4). For this purpose, the 21 blank samples which had been examined during the validation study were used. The noise of the ion trace employed for quantification purposes was calculated in the time-window where the analyte was expected to elute. The CC_αs determined by this method are far below the concentration for which an identification is still possible for nearly all analytes. They are even considerably lower than those determined by variant a. The only exception to this is IPZ. As discussed above, IPZ is strongly matrix-disturbed. Due to this matrix peak a concentration can be determined in the place where the analyte is expected, which differs considerably from the other means of the noise. As a consequence the value determined like this is not meaningful. The results of variant b show that the frequently led critical discussion on the transferability of the blank reading proceeding from mass spectrometric techniques is indeed justified.

As a consequence, a variant c, a modification of variant a was applied. Here, the established matrix calibration curve is not extrapolated linearly, but in parallel to the *x*-axis, in order to simulate a worst-case scenario, where lower concentrations do not produce lower signal responses anymore. This kind of proceeding was firstly introduced by Julicher et al. in 1998 [16]. The *y*-intercept determined like this is used for the further calculation of CC_α, which from there on is continued in analogy to variant a. It could be shown that the CC_αs determined by this method correspond very well to the lowest still identifiable fortification levels established in preliminary tests (Table 4).

The CC_β was calculated with the help of the within-laboratory standard deviation of the respective lowest concentration level. Additional analytical series with samples fortified at the concentration of CC_α were not carried out for this purpose. This proceeding is justified if, like in this case, the reproducibilities of the three concentration levels do not differ significantly.



(a)



(b)

Fig. 4. (a) Matrix blank (turkey muscle) for RNZ and HMMNI respectively; (b) 0.5 μg/kg RNZ in turkey muscle, internal standard D_3-RNZ at 5 μg/kg; (c) 0.5 μg/kg RNZ (0.64 μg/kg HMMNI) in a standard sample, internal standard D3-RNZ at 5 μg/kg.

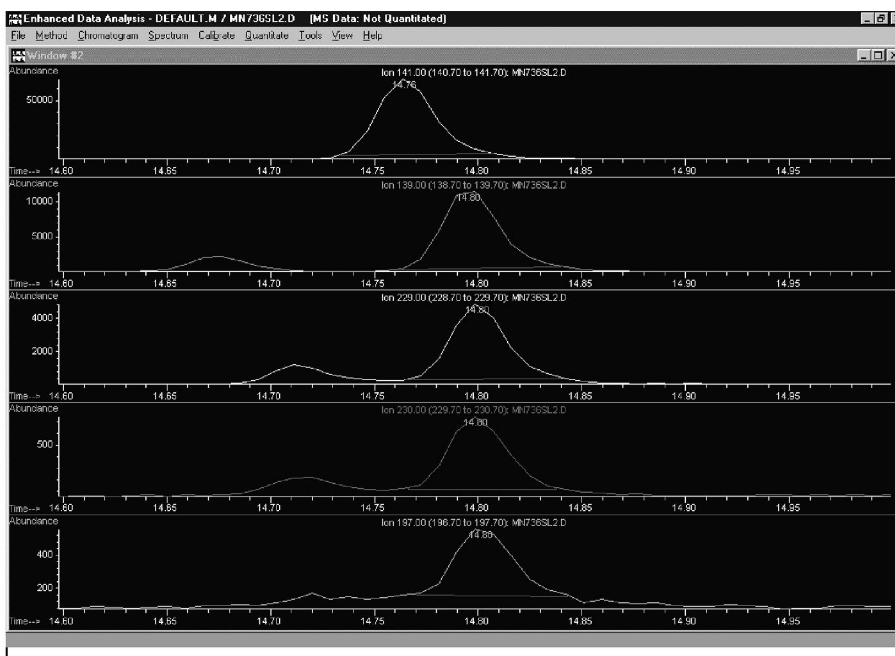


Fig. 4. (*continued*)

In the example presented the calculation of the decision limit CC_α according to variant c delivers the most reasonable results. When applying variant a or c it is advisable to choose the lowest concentration level of the matrix calibration curve in such a way that the confirmation of the substance can be expected with a certain degree of certainty. For this purpose comprehensive pre-investigations have to be carried out, as mentioned above.

3.6. Robustness and selectivity

The method can be considered robust, since different matrices (turkey muscle and swine muscle of different animals) in different states (deep-frozen, fresh, 'not too fresh', stored deep-frozen) of different origins (supermarket, own test animal holding) were used for the validation. Moreover, the analytical series were carried out by different analysts.

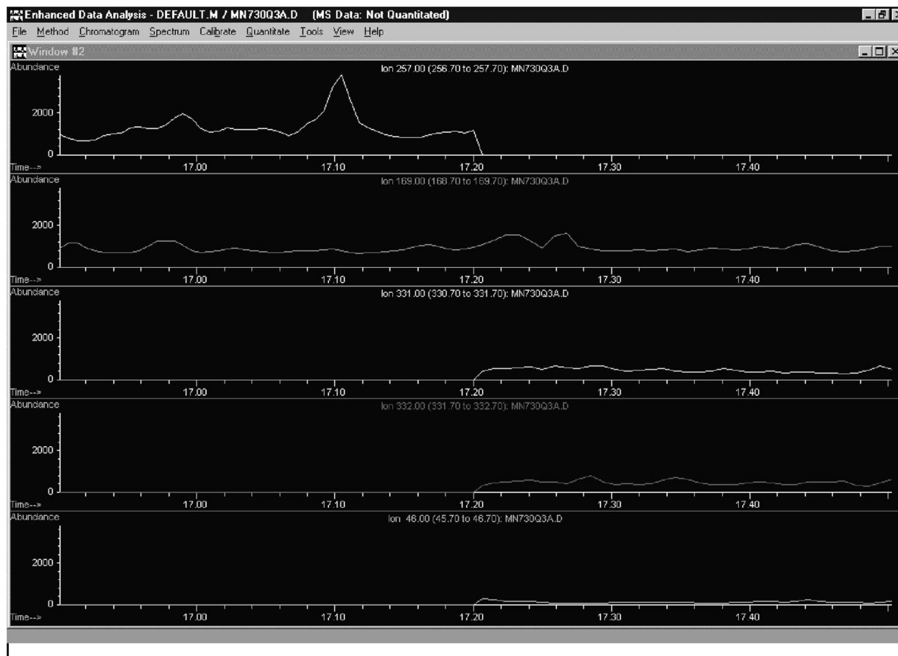
The selectivity of the method is given because structurally related compounds or respectively deuterated standards can be separated unambiguously by means of chromatography or spectroscopic detection.

3.7. Inclusion of HMMNI

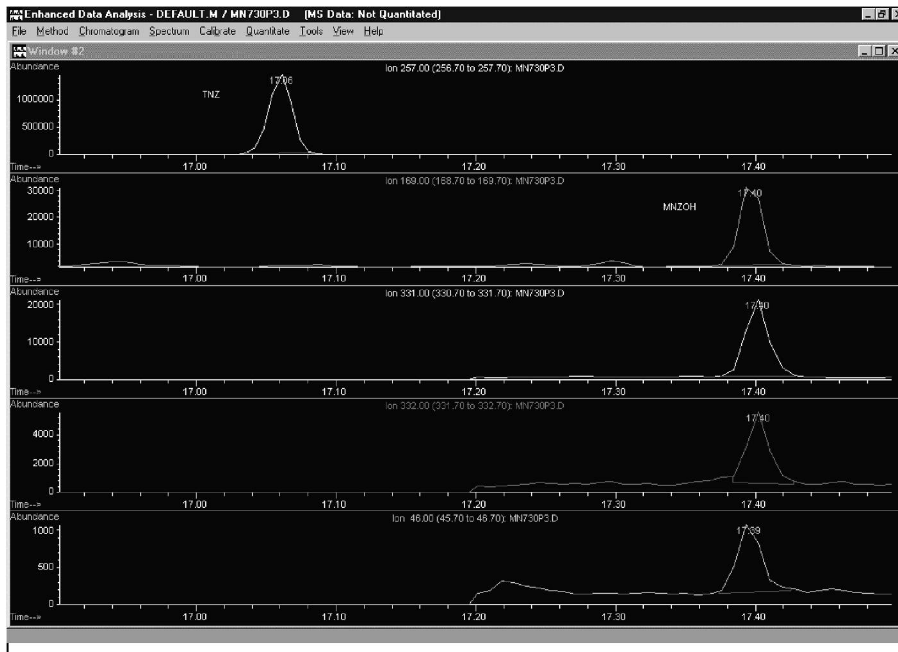
In the chapter on sample preparation and in Fig. 2, the specific role of HMMNI when analysed with GC–MS was described. For this reason it was not possible to include HMMNI in the validation study. Instead a further verification of the applicability of the method presented for HMMNI was carried out by means of an additional fortification study, where HMMNI was added separately to blank material. The measurement results demonstrated that this metabolite produced similar performance data to RNZ. The calculation of these data had been carried out on the basis of the molar content instead of on the basis of mass fraction using RNZ–TMS as a calibrant.

4. Summary

The method presented in this publication is well suited as a screening method for the surveillance of DMZ, RNZ, MNZ, IPZ and their corresponding metabolites due to the simple sample preparation and the low decision limits. Moreover, the presence of



(a)



(b)

Fig. 5. (a) Matrix blank (turkey muscle) for MNZOH; (b) 2 μg/kg MNZOH in a matrix sample, internal standard TNZ at 10 μg/kg; (c) 1.5 μg/kg MNZOH in a standard sample, internal standard TNZ at 10 μg/kg.

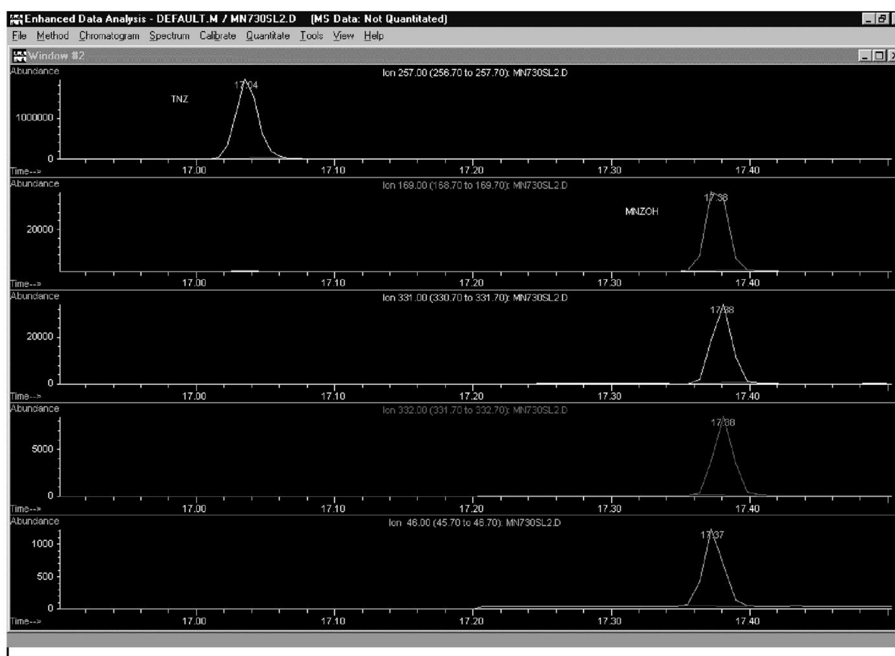


Fig. 5. (*continued*)

the banned nitroimidazoles DMZ, RNZ and MNZ in muscle samples of turkeys and swine can be confirmed with this method. For the confirmation of IPZ and IPZOH an improved sample preparation would be required, which should reliably separate the interfering substances preventing the confirmation and/or offer an increased yield. This would be desirable, as the method is in principle suitable for

Table 3
Repeatability, within-laboratory reproducibility and recovery for nitroimidazoles

	Spike level [μg/kg]	Repeatability [%]	Within laboratory reproducibilty [%]	Recovery [%]
DMZ	2.0/3.0/4.0	4.8/4.7/3.9	9.6/7.3/10.9	101/100/97
D_3-DMZ (IS[a])	(10)			
RNZ[b]	0.5/0.75/1.0	4.4/3.3/2.9	13.7/6.4/12.8	99/97/96
D_3-RNZ (IS)	(5)			
MNZ	2.0/3.0/4.0	13.7/6.4/7.9	15.0/13.3/16.5	94.0/103/102
TNZ (IS)	(10)			
MNZOH	2.0/3.0/4.0	7.6/7.8/11.9	12.2/14.3/20.3	108/114/113
TNZ (IS)	(10)			
IPZ	3.0/4.5/6.0	7.5/7.5/10.8	30.0/22.6/34.4	103/108/118
D_3-IPZ (IS)	(10)			
IPZOH	3.0/4.5/6.0	4.5/6.1/6.5	28.1/9.2/20.4	107/101/105
D_3-IPZOH (IS)	(10)			

[a] IS: Internal standard.
[b] Corresponding to 0.64/0.96/1.28 [μg/kg] HMMNI.

Table 4
Calculation of decision limits

	CC_α [μg/kg] (a)[b]	CC_α [μg/kg] (b)	CC_α [μg/kg] (c)	CC_β [μg/kg] (d)	Curve equation[a]	r[a]
DMZ	0.47	0.13	2.5	2.8	$y = 0.1136x + 0.00059$	0.9989
RNZ	0.17	0.06	0.65	0.76	$y = 0.3468x + 0.01016$	0.9994
MNZ	0.65	0.03	2.5	3.0	$y = 0.0627x + 0.00040$	0.9974
MNZOH	0.59	0.12	2.8	3.2	$y = 0.0109x + 0.00073$	0.9998
IPZ	2.3	2.6	5.2	6.8	$y = 0.1147x + 0.1374$	0.9974
IPZOH	2.1	0.4	5.3	6.8	$y = 0.1017x + 0.0228$	0.9957

[a] Representative curve equation and correlations of one of the three matrix calibrations.
[b] Mean of the three matrix calibrations. (a) From the calibration curve according to the Revision of Commission Decision 93/256/EC, $CC_\alpha = 2.33*$ intra-laboratory reproducibility (SD_{wIR}) of the measurement values/gradient of the calibration curve; (b) according to Darft Revision of Commission Decision 93/256/EC, matrix blank procedure; (c) calibration curve procedure according to an extrapolation of the calibration curve parallel to the x-axis; (d) CC_β calculated as $CC_\alpha + 1.64*$ intra-laboratory reproducibility (SD_{wIR}) of the lowest concentration level, calculated from (c).

the confirmation of these substances in the examined concentration range.

The requirements of the forthcoming EC decision on the quality criteria of analytical results can be implemented with a justifiable amount of analytical work as regards the validation of methods. The comparison of the different calculation procedures (a to c) demonstrated that there was a difference of up to a factor five in the calculated CC_αs although using the same set of data. In order to achieve the comparability of method performance levels and to further harmonise the official residue control of veterinary drugs on a European scale, a standardised procedure for the calculation is advisable. For the validation study presented the calculation of CC_α according to variant c produced the most reliable results. Further validation studies taking into account the revised criteria have to be performed before a decision can be made on whether one-way of proceeding is to be preferred in general.

5. Nomenclature

Decision Limit (CC_α) The Decision Limit is the limit from which on it can be decided that a sample is truly violative with an error probability of α. In the case of banned or unauthorised substances the Decision Limit is the lowest concentration level at which a method can discriminate with a statistical certainty of $1-\alpha$ whether the identified analyte is present. In the case of substances with an established MRL, this means that the Decision Limit is the concentration, above which it can be decided with a statistical certainty of $1-\alpha$ that the identified analyte content is truly above the MRL.

Detection capability (CC_β) This is the smallest content of the analyte that may be detected, identified and/or quantified in a sample with an error probability of β. The β-error should be less than or equal to 5%. In the case of banned or unauthorised substances the detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of $1-\beta$. In the case of substances with an established MRL, this means that the detection capability is the concentration at which the method is able to detect MRL concentrations with a statistical certainty of $1-\beta$.

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