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Note

Determination of detomidine residues in horse meat by negative-ion chemical ionization mass spectrometry

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Detomidine, 4-[(2,3-dimethylphenyl)methyl]-1*H*-imidazole hydrochloride, is a potent sedative for large animals. Through its agonist action on α_2-adrenoceptors it produces analgesia and sedation in very low doses [1]. Pharmacokinetic studies in the two main target species of detomidine, the horse and the cow, have been done by using a highly specific radioimmunoassay (RIA) method [2]. Residue levels of the drug in meat 24 h after the administration of an 80 μg/kg dose were in the range 0–0.8 ppb [3]. The low levels can be attributed to both drug administration as a single dose and to effective elimination by biotransformation [4]. Other than RIA, gas chromatography–mass spectrometry (GC–MS) is the only method available to measure such low concentrations of detomidine. The GC–MS method has been validated earlier for the analysis in serum of medetomidine, a closely related compound, using detomidine as the internal standard [5].

This paper describes a modified GC–MS assay for the quantitative determination of detomidine in meat, to detect possible residues in human food.

EXPERIMENTAL

Materials

Detomidine hydrochloride and the internal standard medetomidine hydrochloride, 4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole hydrochloride, were obtained in pure crystalline form from the synthesis laboratories of Farmos (Oulu, Finland). 2,3,4,5,6-Pentafluorobenzoyl chloride (PFBCl) was from Fluka (Buchs, Switzerland). Other reagents were of analytical grade.

Standard solutions

Dry detomidine hydrochloride (23.9 mg) was dissolved in distilled water and diluted to 1000 ml. A 1.00-ml volume of this solution was diluted to 1000 ml with water, and the final concentration was 20 μg/l (as free base). Medetomidine hy-

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drochloride (23.6 mg) was dissolved and diluted in the same way to give the final concentration of 20 μg/l (as free base).

Spiked meat sample preparation

Fresh meat (from horse back-leg muscles) was obtained from a local slaughterhouse. A piece of muscle (*ca.* 500 g) was homogenized by passing it twice through an ordinary meat grinder. A 5-g amount of the meat pulp was weighed. Two volumes (10 g) of water containing an appropriate amount of standard were added. The mixture was homogenized for 2 min at 1500 rpm in a blade homogenizer and allowed to stand for 15 min. Then 10 ml of 2 *M* HCl were added. Rehomogenization with acid for 2 min yielded a clean extract without interfering lipids. The mixture was centrifuged at 32 000 *g* for 15 min to remove solids. The supernatant was filtered with Gelman Acro LC13 (0.45-μm) filters. Extraction and derivatization were modified from ref. 5. A 1-ml volume of 2 *M* Na_2CO_3 was used to adjust the pH to 12, and 500 pg of medetomidine was added in 25 μl of water.

Standards for quantitative determination were prepared by adding 0–250 μl of the detomidine working standard and the internal standard with water to the meat pulp (5 g) before homogenization. Final concentration of the standard series corresponded 0–1.0 ppb (ng/g) in the original meat samples.

Recovery of the acid extraction from meat

To a 5.0-g minced meat sample (bovine, otherwise horse muscle), 1.0 ml of detomidine standard (including the tritium-labelled tracer) in water and 10 g of 0.9% NaCl were added. Samples with detomidine concentrations of 20, 40, 100, 200, 400, 1000, 2000, 4000 and 10 000 ng/ml were prepared. Each of them was homogenized for 2 min and then divided in two equal parts before any precipitate formed. To the first part 2 ml of water and to the second part 2 ml of 2 *M* HCl were added. The samples were rehomogenized for 2 min and centrifuged at 32 000 *g* for 15 min. The radioactivity of the acid or aqueous extract (supernatant) was measured from three parallel 50-μl samples by a liquid scintillation counter. The recovery was calculated as the fraction of original radioactivity found in the extract.

GC–MS conditions

A Finnigan MAT TSQ 70 mass spectrometer was used in a single-stage mode with a Hewlett-Packard 5890A gas chromatograph equipped with a split–splitless injector. The GC column was a Hewlett-Packard fused-silica capillary column (25 m x 0.20 mm I.D.) packed with cross-linked 5% phenyl methyl silicone (HP Ultra 2). The film thickness was 0.33 μm. The carrier gas was helium with a linear flow-rate of 35 cm/s at the starting temperature. Both the injector and transfer line temperatures were 275°C. The splitless time was 0.75 min. After 1 min at 90°C the GC oven temperature was programmed at 20°C/min up to 275°C, and then

held isothermal for 3.8 min. After each run the column was washed with 1 μl of pure toluene.

In the mass spectrometer chemical ionization (CI) with methane as the reagent gas and negative-ion (NI) detection were used as described earlier [5]. The structures of the molecules and the NICI mass spectra were also presented in ref. 5.

RESULTS AND DISCUSSION

Recovery

Owing to a carrier effect in meat (or any other biological sample) meaningful results for the extraction efficiency could not be obtained by comparison with standards prepared in water. Instead, the recovery of radioactively labelled detomidine was tested in the following way.

The added amount of tritium-labelled detomidine per millilitre of added water in the final homogenate was 166 700 dpm. The activity found in the aqueous extract by scintillation counting, as the mean ± S.D. of the nine tested concentrations (20–10 000 ng/g), was 160 700 ± 5680 dpm, *i.e.* 96.4 ± 3.4% of the added amount. This shows that hydrochloric acid effectively extracts detomidine from homogenized muscle. By contrast, 77 880 ± 10 080 dpm or 46.7 ± 6.0% of the original amount was found in the extract if the acid was replaced by pure water, which shows that about half the amount of detomidine remained bound to the tissue.

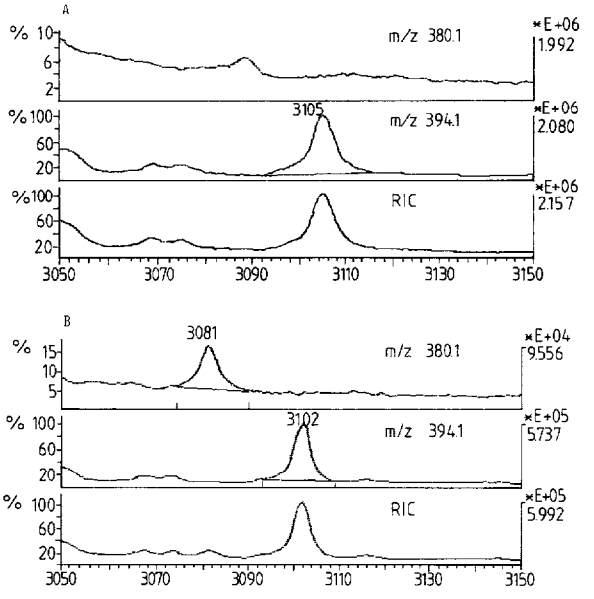

Fig. 1. (A) Selected-ion fragmentograms of extracted blank meat sample. Scan 3105, internal standard. (B) Selected-ion fragmentograms of extracted meat sample spiked with 1.0 ng/g detomidine. Scan 3081, detomidine; scan 3102, internal standard; the lower trace shows the total ion current of the two ions.

Selectivity, linearity and reproducibility

The concentrations in meat, which are in the low ppb range, are too low for obtaining full spectral information without very extensive clean-up and concentration. Instead, selectivity is based on GC separation in combination with selected-ion monitoring. The mass fragmentograms (Fig. 1) are free of interferences from meat. The retention times of detomidine and the internal standard are 8 min 25 s and 8 min 28 s. Known metabolites of detomidine are not extracted or have different retention times and do not interfere.

The calibration curve obtained by assaying spiked meat samples was linear over the measured range. The linear regression equation was $y = 0.003\ 19 + 0.120\ 34\ x$ ($r^2 > 0.96$). It was possible to get a distinguishable signal down to 0.2 ppb.

The reproducibility of repeated quantitation ($n = 5$) was studied at the concentrations of 0.2, 0.4 and 1.0 ppb. The relative standard deviations of the amounts found were 16, 15 and 12% respectively.

CONCLUSION

This GC–MS method is capable of detecting picogram levels of detomidine in meat.

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