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Short Communication

GC–ITD detection and quantitative analysis of Proxazole in cows' plasma and milk

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Introduction

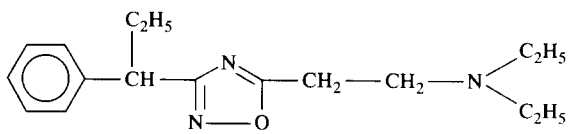
Proxazole [3-(1-phenylpropyl)-5-(2-diaethylaminoaethyl)-1,2,4-oxadiazole] (I) has antitussive, antispasmodic, analgesic, antiinflammatory and antipyretic activities [1–3].

It has veterinary uses against gastritis, infective and non infective gastro-enteritis, ureteritis, cystitis and spastic states with an inflammatory component of the smooth muscles of the digestive and genito-urinary systems.

Proxazole is excreted both in faeces and urine mainly as inactive metabolites.

Owing to its use in veterinary medicine, it is of great importance to detect and measure traces of Proxazole present in milk prior to human consumption and in plasma.

No methods for quantitative analysis of Proxazole are reported in the literature. In this short communication the authors describe a new analytical method based on gas chromatography–mass spectrometry for the quantitation of Proxazole in bovine milk and plasma.



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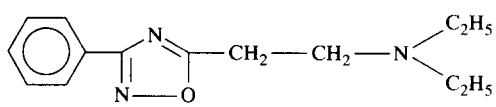

(II)

Experimental

Instrumentation

A Dani 6500 gas chromagraph, coupled with an ITD 800 (Finnigan) mass spectrometer, and equipped with a SPB 20 Supelco fused silica capillary column 0.32 mm in inner diameter, 30 m in length, and a split–splitless injector heated at 250°C was used. The temperature program was as follows: 2 min at 80°C, then 10°C min^{-1} to 220°C, and finally 20 min at 220°C. Carrier gas was helium at 0.7 atm.

Standard solutions

Of Proxazole citrate 1.16 mg, corresponding to 0.695 mg of the free base of Proxazole, were dissolved in 10.0 ml of H_2O giving the standard solution M1; 2.0 ml of M1 were diluted to 10.0 ml with H_2O giving M2 solution; 1.0 ml of M1 was diluted to 25.0 ml with H_2O giving M3 solution; 1.19 mg of Oxolamine (II) citrate (internal standard) were dissolved in 10.0 ml of H_2O giving S1 solution; 2.0 ml of S1 were diluted to 10.0 ml with H_2O giving S2 solution.

Preparation of calibration curves for analysis of Proxazole in plasma and milk

Plasma. To four 3.0 ml plasma samples in 10.0 ml centrifuge tubes were added 0.3, 0.15,

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0.075 ml of M2 and 0.1 ml of M3, respectively, thus preparing solutions containing Proxazole free base at 1.39, 0.695, 0.347 and 0.093 μg ml^{-1} of plasma. To each sample was then added 0.15 ml of S2 solution and, after thorough mixing, 0.1 ml of 10% Na_2CO_3 and 1.5 ml of CH_2Cl_2 (C. Erba RS pesticide grade) were added. The tubes were shaken for 1 min using a Vibromixer and then centrifuged for 10 min. The emulsion thus formed was broken using a glass rod and then the tubes were centrifuged for a further 5 min. The organic layer was sucked out with a Pasteur pipette and applied to a 6 mm i.d. glass column containing 2 g of anhydrous Na_2SO_4. The extraction procedure was repeated three times. The organic extracts were pooled, evaporated to dryness under a dry Nitrogen stream and then dissolved in 0.1 ml of CH_2Cl_2; 1 μl was injected into the gas chromatograph.

Milk. To four 10.0 ml milk samples were added 0.2, 0.1, 0.05 ml of M1 and 0.05 ml of M2, respectively, thus preparing solutions containing Proxazole free base at 1.39, 0.695, 0.347 and 0.07 μg ml^{-1} of milk, and then to each sample 0.1 ml of S1. After thorough mixing 0.4 ml of 20% aqueous citric acid and 5.0 ml of CH_3OH (C. Erba, HPLC grade) were added. The samples were mixed again and then filtered through a fluted filter paper to remove the coagulum. Of the filtrate 4.5 ml (corresponding to 3.0 ml of milk) were rendered alkaline by the addition of 0.2 ml of 10% Na_2CO_3 and then extracted as described for plasma (in this case no emulsion was present).

Results

Figure 1 shows the total ion chromatogram (completely identical to the chromatogram of

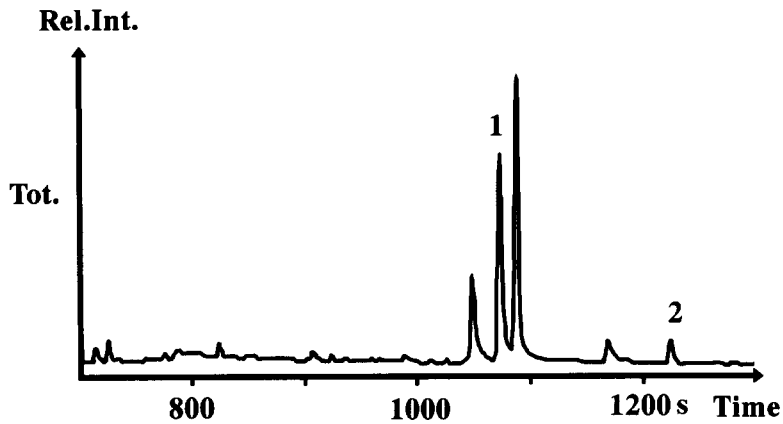

Figure 1
Chromatogram of an extract of plasma containing 0.093 μg ml^{-1} of Proxazole free base (2) and 1.19 μg ml^{-1} of Oxolamine citrate (1). Conditions as described in Experimental.

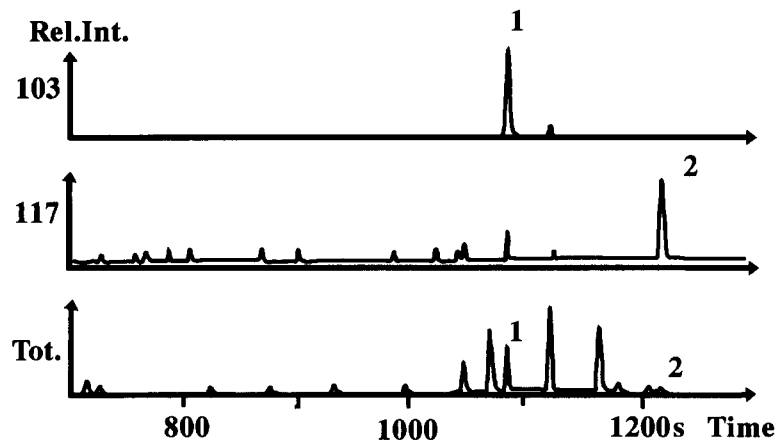

Figure 2
Chromatogram of a milk sample containing 0.070 μg ml^{-1} of Proxazole free base (2) and 1.19 μg ml^{-1} of Oxolamine citrate (1). Conditions as described in Experimental.

Table 1
Relative intensity of the 10 major ions in the mass spectra of Proxazole and Oxolamine

Compound	Ions									
Proxazole	51	58	63	86	89	90	116	117	118	145
	(17)	(28)	(13)	(21)	(15)	(20)	(60)	(100)	(13)	(29)
Oxolamine	50	51	52	56	58	76	86	103	119	131
	(22)	(21)	(12)	(16)	(32)	(38)	(96)	(100)	(14)	(13)

the same sample obtained with a FID detector) of an extract of plasma to which 0.093 μg ml^{-1} (93 ppb) of Proxazole had been added; the signal to noise ratio for the peak of Proxazole was better than 20:1 and method sensitivity was estimated as better than 10 ppb with a signal to noise ratio of 2:1.

Figure 2 shows the total ion chromatogram (TOT) of a milk sample containing 0.07 μg ml^{-1} (70 ppb) of Proxazole. As milk contains many more volatile compounds than plasma, in order to obtain comparable sensitivity one must use selected ion monitoring (SIM). Figure 2 thus shows the SIM trace for the 103 *m/z* ion corresponding to the base peak of the mass spectrum of Oxolamine and the SIM trace for the 117 *m/z* ion corresponding to the base peak of the mass spectrum of Proxazole (see also Table 1).

Using the technique described in this paper sensitivity was better than 10 ppb.

The recovery of Proxazole by the extraction method used was verified by HPLC by comparison with aqueous solutions containing 5.0 μg ml^{-1} of Proxazole citrate, and was found to be quantitative.

Linearity of response was optimum in the range studied: the plots of the sample area/internal standard area ratio vs sample concentration had regression coefficient (r) of 0.990 for plasma and 0.999 for milk.

Reproducibility of the method was assayed on 5 independent 10.0 ml milk samples to which 0.347 g of Proxazole had been added, yielding a relative standard deviation of 4.4%.

The method here described for Proxazole can be used without modification for quantitative analysis of Oxolamine using Proxazole as internal standard.

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