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Note

Determination of benoxaprofen in rat feed by gas chromatography–mass spectrometry

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The anti-inflammatory agent benoxaprofen[1], 2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid (I), and methods pertaining to its raw material assay are discussed elsewhere[2–4]. This report describes the determination of I at levels of less than 0.1% in rat feed samples prepared for toxicological studies.

The basic rat diet contained ground oats, flour and smaller amounts of fish meal, milk products, vitamins and minerals. The assay method was based upon solvent extraction and column chromatographic clean-up techniques, followed by methylation of the carboxylic acid group and gas chromatographic quantitation. Although gas–liquid chromatography (GLC) alone, with flame ionization detection, was used to analyze early batches of rat feed, its use on subsequent batches, even with electron capture detection, was unsuccessful owing to interference from co-extracted material.

However, the problem was solved by the application of quantitative gas chromatography–mass spectrometry (GC–MS) to the analysis of solutions of the methyl ester of benoxaprofen, and methyl 2-(3-trifluoromethylphenyl)-α-methyl-5-benzoxazoleacetate (II), the internal standard. Utilization of the multi-ion detector (MID) facility of the mass spectrometer provided the quantitation of the eluted methyl esters by measuring the ion currents due to two selected ions, one characteristic of each compound. The advantage of the MID technique in minimizing interference from co-extracted material was evident, as a sensitive response was obtained from the monitoring of the selected ion at the characteristic retention time of methylated I or II. The final method adopted therefore possesses high specificity and is likely to be applicable to rat diet batches of varying composition. In the absence of mass spectrometry fairly extensive changes in clean-up may be necessary for different batches of diet.

EXPERIMENTAL

Extraction

A 20-g sample of dosed rat feed was extracted with 100 ml of acetone, the extract evaporated to dryness, and the residue made up to volume in chloroform (2.0, 5.0 and 10.0 ml for 30, 100 and 300 ppm feeds, respectively). A 1.0-ml aliquot was chromatographed on 90–200 μm silica using chloroform as preliminary eluent to remove interfering material, before elution of benoxaprofen with 5% methanol in chloroform and evaporation of the eluate to dryness.

A 20-g sample of undosed rat feed was taken through the procedure to measure the interference from diet components, and a similar sample, spiked with 1.0 ml of an acetone solution of benoxaprofen at 0.5 mg/ml, was used to establish the standard recovery for a set of determinations. (Intermediate dilution to 5.0 ml with chloroform was used.)

The residue from the column eluate was treated with excess of ethereal diazomethane and evaporated to dryness. 1.0 ml of II (0.04 mg/ml in chloroform) was added to the residue. Standard solutions of the methyl ester of I from 0.02 to 0.10 mg/ml were prepared in chloroform containing 0.04 mg/ml of II.

Gas chromatography–mass spectrometry

Solutions were assayed on a LKB 9000S GLC–MS apparatus equipped with a four-channel Altema AL5 multi-ion detector. Chromatography was performed on a 3 ft. × 4 mm I.D. glass column packed with 2% XE-60 on Gas-Chrom Q (100–120 mesh), using a column temperature of 210°, a 2-μl on-column injection and a helium flow-rate of 20 ml/min. The Ryhage jet separator was maintained at 265° and the ion source at 270°. The ionizing voltage was set at 20 eV and the accelerating voltage at a nominal 3.5 kV. The required ions were brought into focus by switching the accelerating voltage with the Altema MID unit at a fixed magnetic field. The signal from each ion was recorded on a four-channel visigraph FR3017 direct-reading oscillograph.

RESULTS AND DISCUSSION

A linear standard calibration curve was obtained on plotting the ratio of the selected ion intensities (*m/e* 256 for methylated I over *m/e* 290 for II) against weight of I. On occasions unacceptable drift of the magnetic field after several hours necessitated the preparation of a fresh calibration curve.

In the absence of a deuterium-labelled standard or homologue of I the chemically similar compound II was chosen for the MID analysis. Both methyl esters are particularly suitable for MID analysis as a large percentage of their total ion currents are carried by only two ions (Fig. 1), the molecular ion, and the base ion resulting from simple scission of the ester group (a loss of 59 mass units). The base ions were used for quantitation, the chlorine isotope ratios for I confirming the absence of interference from rat diet components. The selectivity of the MID analysis enables samples of methylated I and II to be eluted within a short time (3 min) allowing a fast turnover of assays.

The assays of benoxaprofen in rat feed samples taken from different portions

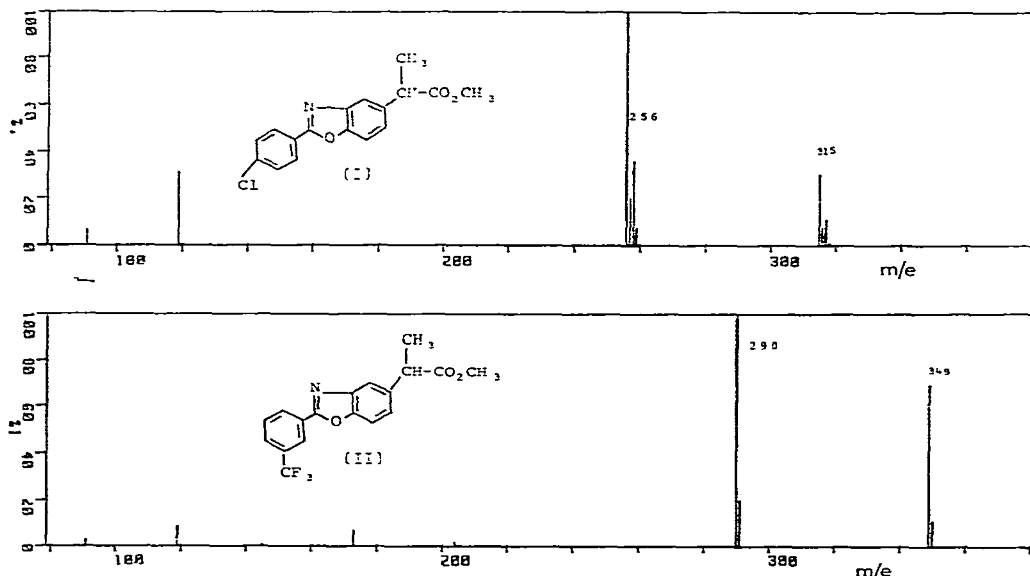

Fig. 1. Mass spectra of the methyl ester of 2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid (I) and methyl 2-(3-trifluoromethylphenyl)-α-methyl-5-benzoxazoleacetate (II).

TABLE I

ASSAYS OF BENOXAPROFEN IN TYPICAL BATCHES OF RAT FEED

Calculated using standard recovery results of 93.0% and 90.5% (average recovery of 91.8%).

Region of the blend	*Mix (ppm)*		
	30	*100*	*300*
Top	29	105	302
Middle	30	106	309
Bottom	31	108	298

of a typical blended batch are shown above (Table I). Results, corrected for the average standard recovery of 91.8%, are close to the formulated values, suggesting satisfactory assay accuracy and precision, as well as homogeneity of the batch.

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