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Note

Quantitative determination of naproxen in formulated rat feed by gas chromatography–mass spectrometry and gas chromatography–flame-ionization detection

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Good laboratory practices require quality control of the drug–feed mixtures fed to animals on long term toxicity studies. In the past few years an increasing number of papers have been published which described methods for the analysis of a wide variety of compounds in feed[1,2]. These procedures employed a range of techniques including UV spectrophotometry, gas chromatography (GC) and high-performance liquid chromatography (HPLC).

Drug dosage is dependent upon body weight so drug concentrations in feed are low for the young rats and increase in proportion as the rat matures, typical concentrations being in the range 0.002–0.05% of naproxen in the feed. It is essential with this type of feeding approach to toxicity studies that the homogeneous mixing of the drug in feed and its stability therein be constantly monitored.

Once the drug has been mixed with feed and subjected to the repelleting process it is necessary to use polar solvents for complete extraction. None of the currently available methods for the determination of naproxen in plasma or urine[3–6] could be applied to feed either for efficiency of extraction or their ability to clean up the tremendous amount of background interference.

This paper describes the analysis of naproxen, (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, from feed using either GC–mass spectrometry (MS) or GC–flame-ionization detector (FID) techniques. The two techniques between them provide the necessary specificity, sensitivity and a method for routine analysis of large numbers of feed samples. Application of the GC–MS method to demonstrate the homogeneity and stability of naproxen in repelleted formulated rat feed is described.

EXPERIMENTAL

Reagents

Diazomethane was prepared from N-methyl N-nitroso-*p*-toluene sulfonamide as per the instructions on the manufacturers label (Diazald; Aldrich, Milwaukee, WI, U.S.A.). All solvents used were either glass distilled or analytical reagent grade.

Instrumentation

The gas chromatograph–mass spectrometer (Finnigan Model 3200) was equipped

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with a chemical-ionization source and a Model 6000 data system. Methane was used as the carrier gas (20 ml/min) and also as chemical ionization reagent gas, the source pressure being maintained at approximately 1 torr. The column was 150 cm × 2 mm I.D. glass packed with 3% OV-1 in 100–120 mesh Chromosorb W.

Column, injection port, transfer oven and source temperatures were maintained at 150, 260, 260 and 120°C, respectively. The mass spectrometer was operated in the selected ion recording mode, monitoring ions at 231 and 245 a.m.u.

The gas chromatograph (Model 2100, Varian, Walnut Creek, CA, U.S.A.) was equipped with an FID. The column was 180 cm × 2 mm I.D. glass packed with 3% SP-2401 on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). Injector, column and detector were maintained at 275, 150 and 300°C, respectively.

Procedure

Method A: GC–MS. To 25 grams of well ground and mixed sample in a 500-ml erlenmeyer flask was added the appropriate amount of internal standard, 6-methoxynaphthylacetic acid in methanol.

Extraction was achieved by stirring in 240 ml of acetonitrile–water (1:1) for 20 min at 35°C. After centrifugation, a portion of the supernatant, approximately 3 ml, was transferred to a separatory funnel. A 9-ml volume of acetate buffer (pH 3.4) was added and the solution extracted with 10 ml of ether. The organic layer was filtered through a plug of anhydrous sodium sulfate and evaporated to dryness at 40°C under nitrogen. Diazomethane in ether (0.8 ml) was added and the methylation reaction was allowed to proceed for 5 min at room temperature. After evaporation of excess reagent under nitrogen, the residue was dissolved in 200 μl of methanol and 2–5 μl were injected for analysis in the GC–MS system.

Method B: GC–FID. Initial extraction from the feed was similar to method A with 6-ethoxy naphthyl acetic acid being used as the internal standard. A 2-ml volume of the supernatant was acidified with 2 ml of 2 *N* HCl, extracted with 10 ml of dichloromethane–hexane (3:7) and centrifuged. The organic layer was evaporated to dryness at 40°C under nitrogen. The residue was dissolved in 3 ml of 1 *N* sodium hydroxide, washed with 7 ml of dichloromethane, acidified with 1.7 ml of 2 *N* HCl and extracted with 5 ml of ether. The residue after evaporation of the ether was methylated in the same manner as in method A. The final residue was dissolved in 100 μl of carbon disulfide and 1–2 μl injected for analysis on the gas chromatograph.

RESULTS AND DISCUSSION

Naproxen is a polar compound containing a carboxylic acid function which necessitates the use of very polar solvents to achieve complete extraction. This, of course, tends to elute more background interference from the very complex and variable feed matrix. The GC–MS technique, due to its greater specificity, did not detect much of the background interference thereby allowing the development of a simple procedure in a short time. The specificity afforded by the GC–MS stems from the use of chemical ionization and selected ion monitoring techniques. Chemical ionization spectra are usually simpler than electron impact spectra and therefore give rise to less interference from endogenous compounds. The selected ion monitoring technique provides sensitivity and specificity since only the quasi-molecular ions of

naproxen and the internal standard are actually monitored so lessening the chance of background interference. In fact GC analysis of the extracts did show background interference that was not visible on the GC–MS ion chromatograms. The chemical ionization mass spectra obtained for the methyl esters of naproxen and the internal standard are shown in Fig. 1. Typical ion chromatograms obtained by monitoring the quasi-molecular ions of $MH^+ = 245$ for naproxen and $MH^+ = 231$ for the internal standard are shown in Fig. 2. The sensitivity necessary to measure the lowest required level of 0.002% could easily be obtained and calibration curves prepared in the range of 0.001 to 0.03% were linear.

The data presented in Table I show that the drug is accurately formulated and homogenously distributed in the feed. Assay results were usually within 5% of the label strength. In order to determine homogeneous mixing of the drug in feed, samples

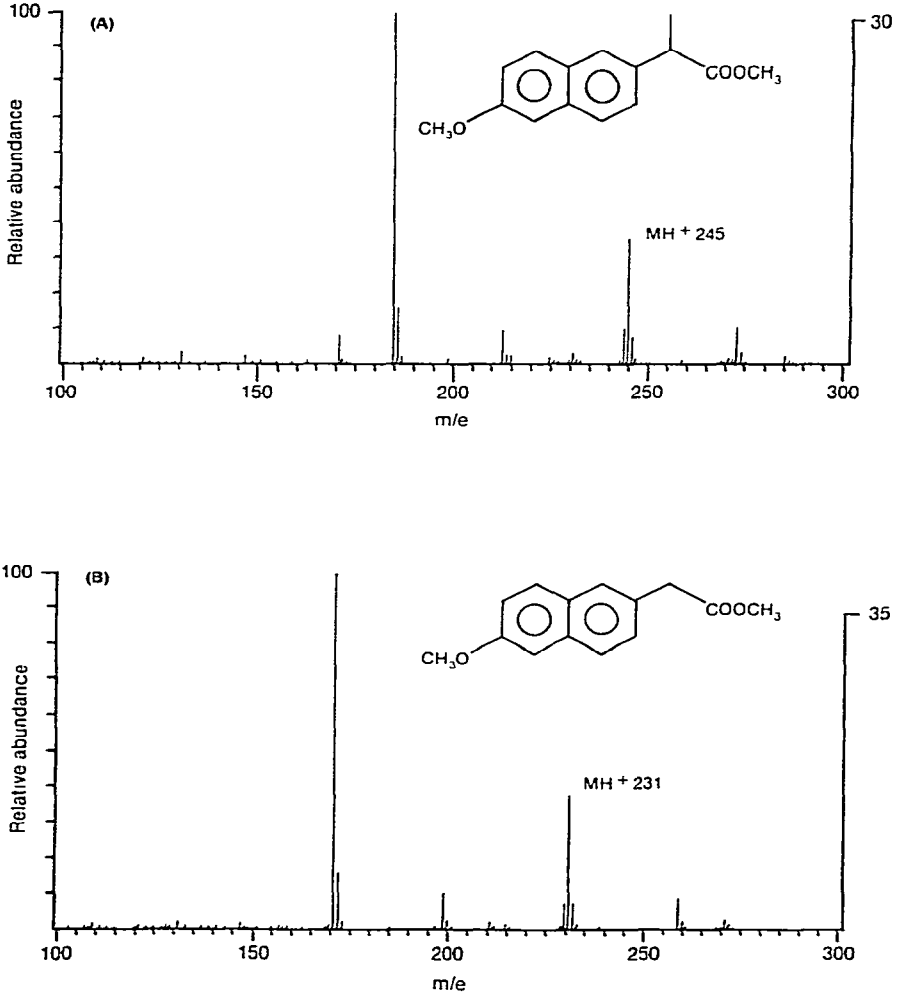

Fig. 1. Methane chemical ionization mass spectra of the methyl esters of naproxen (A) and internal standard (B).

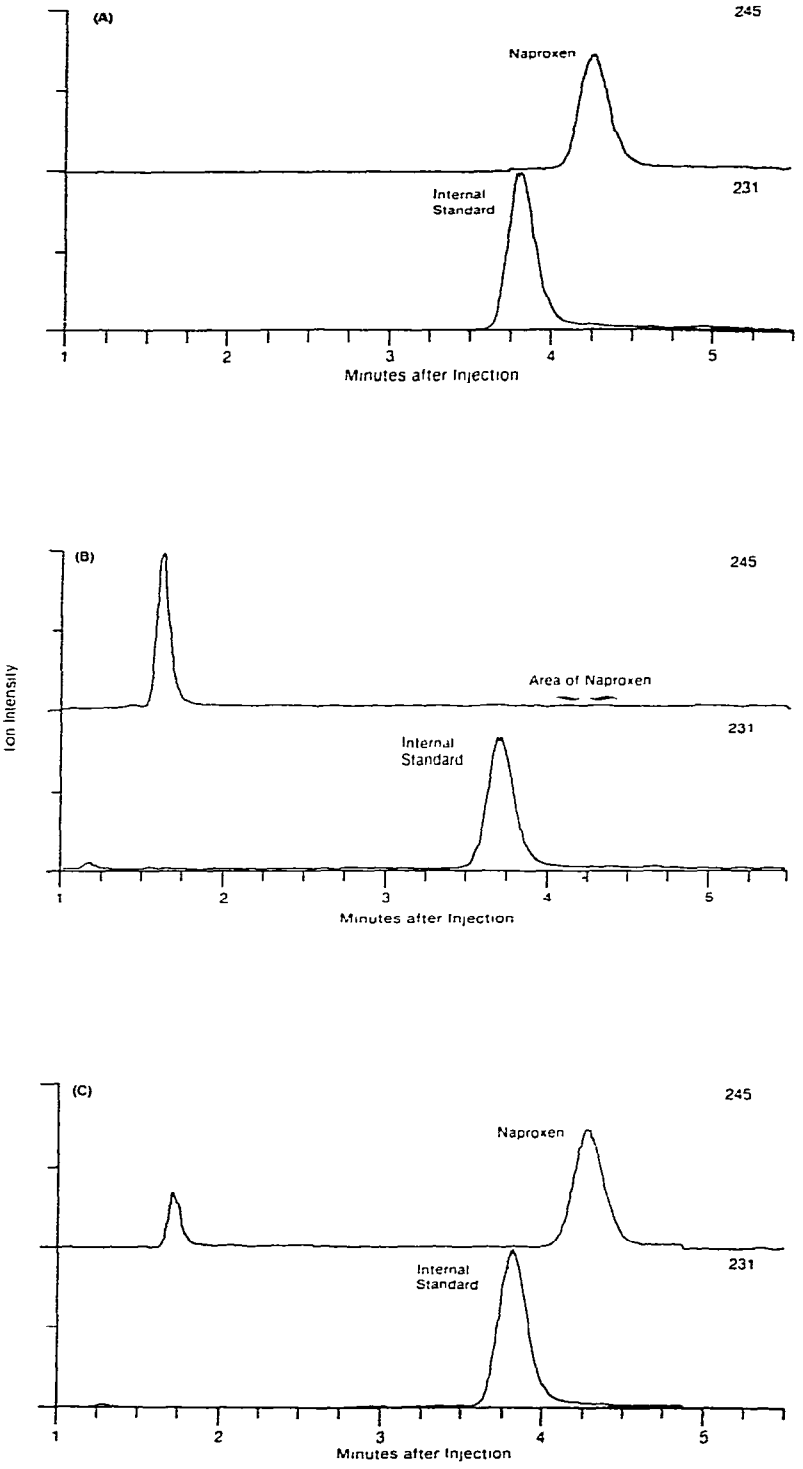

Fig. 2. Selected ion chromatograms obtained for method A. (A), reference standards of the methyl esters of naproxen and internal standard; (B), blank feed sample spiked with internal standard; (C), formulated feed sample spiked with internal standard.

TABLE I

ASSAY RESULTS INDICATING ACCURACY OF FORMULATION AND HOMOGENEITY OF NAPROXEN IN RAT FEED

The feed was repelletized after formulation and samples taken for analysis during the beginning, middle and end of the pelleting run to check homogeneity.

Sample	*Label strength (%)*	*Assay value (%)*
Beginning	0.0040	0.0037
Middle	0.0040	0.0038
End	0.0040	0.0039
Beginning	0.0080	0.0078
Middle	0.0080	0.0077
End	0.0080	0.0078
Beginning	0.0120	0.0117
Middle	0.0120	0.0116
End	0.0120	0.0118
Beginning	0.0053	0.0051
Middle	0.0053	0.0051
End	0.0053	0.0050
Beginning	0.0159	0.0154
Middle	0.0159	0.0153
End	0.0159	0.0158

TABLE II

ASSAY RESULTS INDICATING THE STABILITY OF NAPROXEN IN FORMULATED FEED

Sample	*Label strength (%)*	*Initial assay (% of l.s.)**	*1 Week room temp. (% of l.s.)*	*3 Week room temp. (% of l.s.)*	*4 Week room temp. (% of l.s.)*	*1 Week 37°C (% of l.s.)*
"Stability"	0.0100	100	101			101
No. 850	0.0053	96		98		
No. 851	0.0106	95		95		
No. 852	0.0159	96		93		
No. 864	0.0075	87			89	
No. 865	0.0150	96			95	
No. 866	0.0225	93			96	

* l.s. = Label strength.

of the feed were taken for analysis at the beginning, middle and end of the repelleting process.

It is an essential requirement that the drug be stable in the feed for the period elapsing between formulation and utilization. The data presented in Table II show that naproxen levels were unchanged when analyzed one month later.

As the rat toxicity study progressed we adapted the GC–MS method to the more routine and economically attractive GC–FID technique. To achieve this end, it was necessary to increase the number of clean-up steps, as outlined in method B. Typical gas chromatograms are shown in Fig. 3. A slightly modified procedure was eventually used to monitor the remainder of the two-year rat toxicology study.

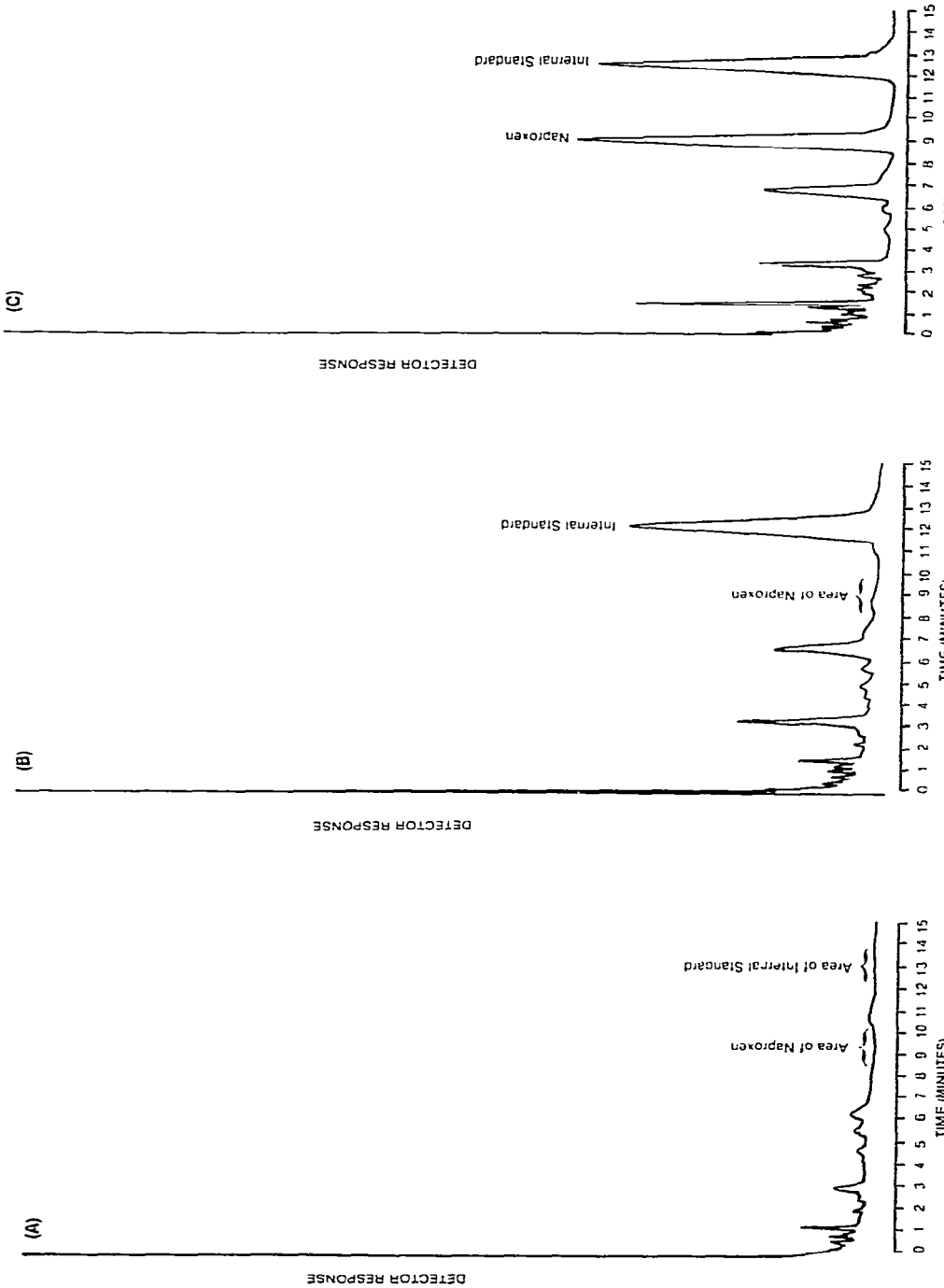

Fig. 3. Gas chromatograms obtained for method B. (A), blank feed sample; (B), blank feed sample spiked with internal standard; (C), formulated feed sample spiked with internal standard.

GC–MS is an expensive technique when compared with GC and HPLC and would not normally be the method of choice for economic reasons. However, it should be remembered that when the instrument is available it can be used to considerable advantage as a highly specific detector as well as in its more traditional role as a highly sensitive technique.

In conclusion, analytical procedures have been presented which covered all the feed assay requirements for a 2-year chronic toxicity study for naproxen in rats.

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