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Journal of Chromatography B, 676 (1996) 53–59

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Evaluation of a benchtop ion trap gas chromatographic–tandem mass spectrometric instrument for the analysis of a model drug, tebufelone, in plasma using a stable-isotope internal standard

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Received 11 August 1995; revised 28 September 1995; accepted 29 September 1995

Abstract

The performance of a benchtop GC–ion trap MS–MS instrument, the Varian Saturn 4D, was evaluated for the analysis of a model drug, tebufelone, in plasma. The sample preparation scheme was designed to provide a highly complex extract with matrix-derived interferences eluting near and at the retention time of tebufelone and its stable-isotope-labeled analog. The performance of the ion trap in the selected-reaction-monitoring mode was evaluated and also compared with results obtained on a benchtop GC–MS linear quadrupole instrument operated in the selected-ion-monitoring mode. The ion trap, operated in the selected-reaction-monitoring mode, was found to provide a higher degree of selectivity for the analysis of tebufelone. The increased selectivity obtained on the ion trap operated in the selected-reaction-monitoring mode resulted in superior accuracy and precision, as well as a lower limit of quantitation relative to that obtained by the GC–MS analysis. A linear standard curve was obtained over three orders of magnitude and the limit of quantitation for tebufelone in plasma was 100 pg/ml using the GC–ion trap MS–MS instrument.

Keywords: Tandem mass spectrometry; Ion trap GC–MS–MS instrument; Tebufelone

1. Introduction

The determination of drugs in biological matrices plays a central role in the development of modern pharmaceuticals. Data generated from these measurements play a key role in the interpretation of toxicology studies, provides insight into a drug's mechanism of action, helps in the evaluation of structure-activity studies, establishes pharmacokinetic parameters and provides the means for monitoring therapeutic levels of drugs in large populations [1]. Modern pharmaceuticals are becoming increasingly potent, with many drugs exerting their effects at blood concentrations in the ng/ml to pg/ml range. Low therapeutic drug levels, combined with the complex nature of biological matrices, makes the ultratrace analysis of drugs an extremely challenging undertaking. Historically, high resolution gas chromatographic techniques, coupled with a variety of selective and sensitive detectors, have played a central role in the analysis of drugs in various biological matrices. The hyphenated technique of gas

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SSDI 0378-4347(95)00417-3

chromatography–mass spectrometry (GC–MS), used in combination with stable-isotope-labeled internal standards, offers a highly selective, sensitive and accurate means for the ultratrace analysis of drugs in biological fluids. The mass spectrometer used in the selected-ion-monitoring (SIM) mode provides an additional dimension of selectivity to that already obtained from the high resolution capabilities of modern GC capillary columns. However, even with the high selectivity obtainable with GC–MS methodology, endogenous matrix components can still interfere in the ultratrace analysis of drugs.

Tandem mass spectrometry (MS–MS) techniques can provide an additional dimension of selectivity for ultratrace drug analysis. For MS–MS, using a triple quadrupole mass spectrometer, a selected-reaction-monitoring (SRM) scheme is used whereby a parent ion, isolated by the first quadrupole, undergoes collisionally-activated-dissociation (CAD) in a second quadrupole and a daughter ion formed from the CAD process is detected after isolation by the third quadrupole of the instrument [2]. The potential for interferences from endogenous matrix components is thereby greatly reduced. Ultratrace drug analysis methods based on GC–MS–MS approaches have been shown to be highly selective, sensitive and rugged [3,4]. Although the advantages of the MS–MS approach for ultratrace drug analysis are clear, the cost of current tandem instrumentation for gas chromatography, greater than $300 000 per instrument, often makes the utilization of this technology beyond the reach of many laboratories.

The recent introduction of benchtop GC–ion trap based MS–MS technology offers the potential of performing SRM analysis on an instrument priced competitively with current benchtop GC–MS linear quadrupole instruments. The functioning of ion traps in the MS–MS mode differs physically from that of tandem mass analyzer instruments in that the CAD experiment is performed using a single rotationally symmetric mass analyzer which exerts a trapping quadrupole field. Fundamentally, ion traps perform the MS–MS experiment in time, while tandem mass analyzer instruments perform the experiment in space. Several reviews of ion trap MS–MS technology have been published [5,6]. Previous work with ion trap instruments, operated in the MS and MS–MS mode using co-eluting internal standards, has highlighted a number of limitations of this technology when applied to the ultratrace analysis of drugs in biological matrices [7,8]. Most significantly, the lack of automatic gain control severely limited the linear dynamic range of the instruments.

The purpose of this work was to examine the performance of a new benchtop GC–ion trap MS–MS instrument for the analysis of a model drug in a biological matrix. Additionally, the performance of the GC–ion trap MS–MS instrumentation was compared to that of a benchtop GC–MS linear quadrupole instrument. Tebufelone (TEB) was chosen as the model drug and rabbit plasma was chosen as a model biological matrix for the evaluation of the ion trap. TEB was chosen as the model compound since its analysis by both GC–MS and GC–MS–MS, using tandem and trap based instruments, had been previously reported [3,7–9]. An extraction procedure was developed that provided matrix interferences both near and at the retention time of TEB to obtain a worst-case scenario for analysis. The selectivity, linear range, accuracy and precision of the benchtop GC–ion trap instrument, operated in the SRM mode, with electron ionization was evaluated in these studies.

2. Experimental

2.1. Chemicals

TEB and [^{13}C,^{18}O]-labeled TEB (IS-TEB), see Fig. 1, were prepared at Procter and Gamble's Miami Valley Laboratories (Cincinnati, OH, USA). Hexane

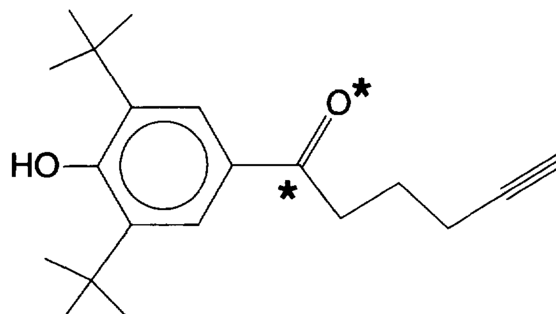

Fig. 1. Structure of tebufelone. The * symbols indicate the position of the [^{13}C] and [^{18}O] isotopic labels.

and 0.1 *M* HCl were from J.T. Baker (Phillipsburg, PA, USA). Rabbit plasma was purchased from Pel-Freez Biologicals (Rogers, AR, USA).

2.2. Preparation of rabbit plasma blank extract

Blank rabbit plasma (1.0 ml) samples in a series of test tubes were each mixed with 0.1 *M* HCl (1.0 ml) and then individually extracted with 2.0 ml of hexane. The individual hexane extracts were taken to dryness under nitrogen at 30°C on a TurboVap (Zymark, Hopkington, MA, USA) and the residue of each tube reconstituted in hexane (0.2 ml). The reconstituted hexane extracts were combined and a portion of the combined extract was used as the blank rabbit plasma sample. The remainder of the combined hexane extract was used to prepare TEB spiked samples as described below.

2.3. Preparation of TEB-spiked rabbit plasma extract

Test tubes containing 2.0 ng of IS-TEB were spiked with aliquots of the appropriate TEB stock solution to give triplicate samples containing 0.27, 2.7 or 27 ng TEB. The solvent was removed under nitrogen and the residue was reconstituted with 0.2 ml of the reconstituted rabbit plasma extract prepared above. The samples were transferred to low-volume autosampler vials and capped with Teflon-lined caps. The spiked samples were prepared in this manner to allow the performance of the instrument to be evaluated without the variability introduced by an actual sample preparation procedure.

2.4. Preparation of TEB standards

Test tubes containing 2.0 ng of IS-TEB were spiked with aliquots of the appropriate TEB stock solution to give a series of standards covering a mass range from 0.1 to 270 ng. The solvent was removed under nitrogen and the residue was reconstituted with 0.2 ml of the reconstituted rabbit plasma extract prepared above. The standards were then transferred to low-volume autosampler vials and capped with Teflon-lined caps.

2.5. Benchtop linear quadrupole GC–MS conditions

A Hewlett-Packard (HP) Model 5890A Series II gas chromatograph, a HP Model 5971A MSD and a Model 7376A autosampler were used with a J&W Scientific (Folsom, CA, USA) DB-5 MS capillary column (30 m×0.25 mm I.D., 0.1 μm film thickness) for the GC–MS analysis. Helium was used as the carrier gas, with a head pressure of 55 kPa. The injection port contained a deactivated 4-mm straight liner packed lightly with silonized glass wool. A 2-μl volume of standard or sample was injected in the splitless mode, with the split vent opening at 1.0 min. The thermal program involved an initial isothermal hold at 100°C, followed by a linear ramp (40°C/min) to 300°C and a final hold at 300°C for 4 min. The injection port and transfer line were held at 250 and 300°C, respectively. For SIM, a dwell time of 100 ms each was used to monitor m/z 248 (TEB) and 251 (IS-TEB), respectively. Full scan mass spectra were obtained by scanning a mass range from m/z 75 to 650. All GC–MS referred to in this report was performed using this linear quadrupole system. No GC–MS work was performed on the ion trap instrument.

2.6. Benchtop ion trap GC–MS–MS conditions

A Varian Model 3400 CX gas chromatograph, a Saturn 4D mass spectrometer and a Model 8200 CX autosampler were used with a J&W Scientific DB-5 MS capillary column (30 m×0.25 mm I.D., 0.1 μm film thickness) for the GC–MS–MS ion trap analysis. Helium was used as the carrier gas with a head pressure of 55 kPa. The injection port contained a deactivated 4-mm straight liner packed lightly with silonized glass wool. The standards and samples were injected and chromatographed exactly as described above for the GC–MS analysis. The injection port and manifold temperatures were held at 250 and 300°C, respectively. The basic MS–MS scheme involved a prescan to set the ionization time followed by an analytical scan. For the prescan, both parent ions of m/z 248 and 251 were isolated in an 8-amu parent mass window (m/z 247–255). This was done by applying a broad band multi-frequency waveform during the ionization period and for a

short "cool time" after the end of the ionization to resonantly eject ions of mass-to-charge ratio above and below the target ion window. Any remaining ions with masses below the target window were removed by resonantly scanning them out of the trap by applying a single frequency dipole field (485 kHz) to the end caps of the trap and moving the operating point of the ion window to q_z=0.91 by increasing the radio frequency (rf) trapping voltage. The remaining ions with masses above the target ion were then removed by lowering the operating point of the ion window to q_z=0.84 coincident with the application of a broadband waveform containing frequencies between 20 kHz and 400 kHz with a 500-Hz spacing. The resulting total ion current obtained during the prescan was used by an automatic gain control algorithm to set the ionization time for the analytical scan. During the subsequent analytical scan the same 8-amu parent window was isolated as described above and subjected to non-resonant excitation to simultaneously generate daughter ions of TEB and IS-TEB at *m/z* 233 and 236, respectively. The resulting daughter ions were sampled using a narrow-range rf scan. The chromatograms for the selected mass transitions were reconstructed post-run from the narrow-range product ion scan data. The exact instrumental MS–MS conditions were: parent mass, *m/z* 250; mass isolation window, 8.0 *m/z*; waveform type, non-resonant; excitation time, 30 ms; excitation amplitude, 40.0 V; ejection amplitude, 20.0 V; broadband amplitude, 30.0 V; low mass daughter, 231 *m/z*; high mass daughter, 238 *m/z*; scan rate, 400 ms; multiplier voltage, 2200 V; A/M amplitude set voltage, 3.0 V; emission current, 90 μA, AGC prescan ionization time, 100 μs, and RF dump value, 650.0 *m/z*.

2.7. Quantitation of TEB in spiked rabbit plasma extracts

The standards were analyzed by GC–MS using the linear quadrupole instrument and GC–MS–MS using the ion trap instrument. The peak-area ratio, TEB peak area/IS-TEB peak area, was calculated for each standard. The peak-area ratio obtained for a standard was then divided into the mass of the standard to generate the response factor for that standard. The mean response factor was then calculated for the standard curve. The mass of TEB in each sample was calculated by multiplying the peak-area ratio obtained for the sample by the mean response factor obtained for the standards.

3. Results and discussion

3.1. Parent and daughter mass spectra

The mass spectra of TEB obtained on the linear quadrupole GC–MS and ion trap instruments are shown in Fig. 2. On the quadrupole instrument a small parent ion was observed at *m/z* 300 and the major ions occurred at *m/z* 233 and *m/z* 248 (Fig. 2A). The structures and origins of the TEB ion fragments has been discussed in detail in previous publications [3,8]. The same major ions were generated on the ion trap, *m/z* 233 and 248, but no parent ion was observed at *m/z* 300 (Fig. 2B). The parent ion *m/z* value selected for MS–MS experiments was the 248 ion. The daughter spectrum obtained for this parent is shown in Fig. 2C. The daughter spectrum was dominated by a major ion at *m/z* 233. Similar spectra were obtained for IS-TEB but the *m/z* values for the major ions were shifted by 3 amu, due to the presence of the ^{13}C and ^{18}O isotopes. The SRM schemes selected for the GC–MS–MS analysis were parent-to-daughter transitions *m/z* 248 to *m/z* 233 and *m/z* 251 to *m/z* 236 for TEB and IS-TEB, respectively. The current software on the Saturn 4D did not allow for the isolation of single mass parent ions but rather isolated ions over a narrow mass range. Therefore, a mass range from *m/z* 247 to 255 actually was used to isolate the parent ions for both TEB and IS-TEB. It should be noted that this type of scan function would not be applicable to situations where common-daughter ions were formed by the analyte and the stable-isotope-labeled internal standard.

3.2. Selectivity

The GC–MS total ion chromatogram obtained for the blank rabbit plasma extract contained a high level of matrix contaminants eluting near and at the retention time of TEB as revealed by the total ion chromatogram (Fig. 3A). GC–MS analysis of the

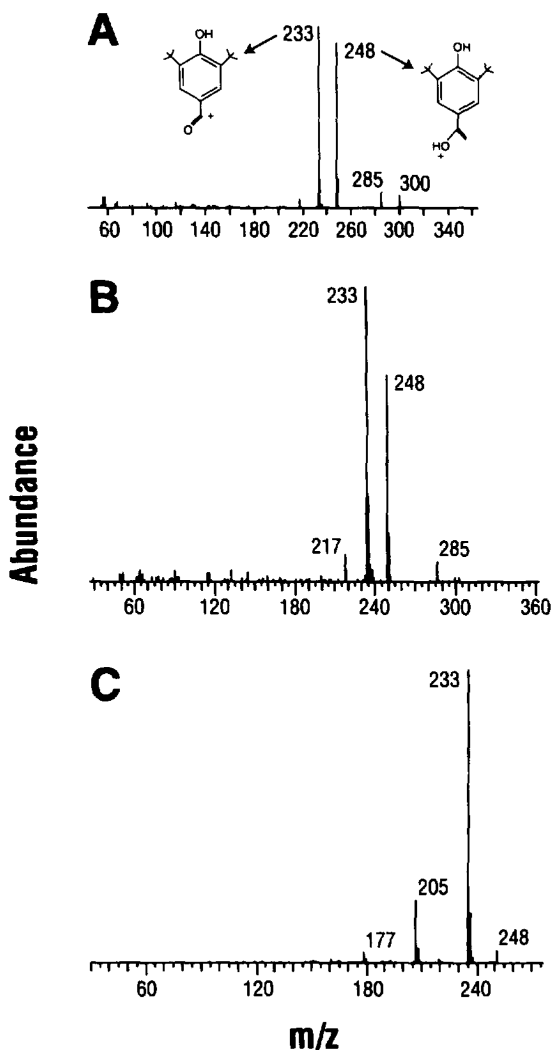

Fig. 2. Mass spectra of tebufelone obtained on: (A) quadrupole mass spectrometer, (B) ion trap based mass spectrometer and (C) daughter spectrum obtained on an ion trap mass spectrometer for m/z 248 parent ion.

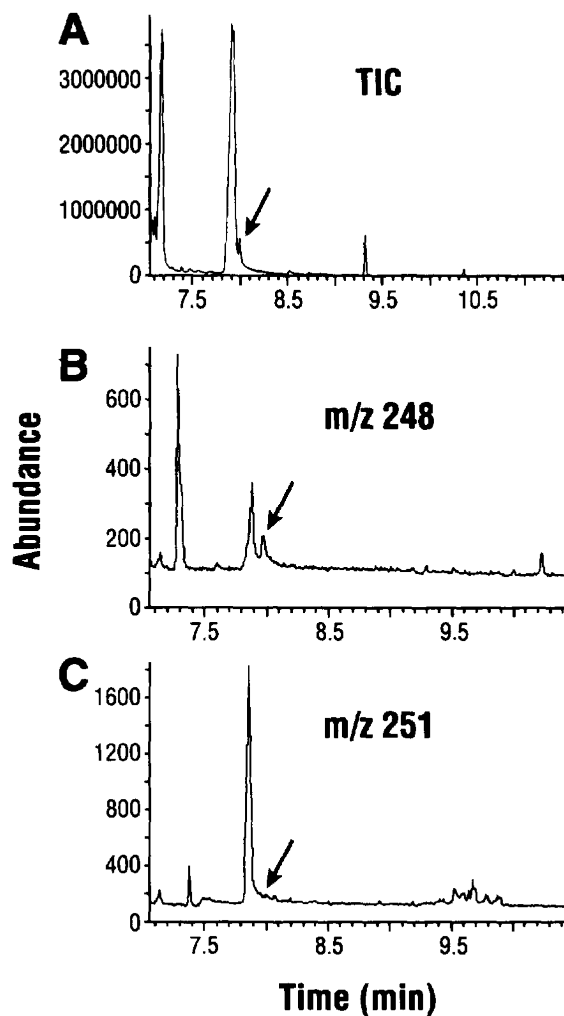

Fig. 3. Analysis of blank rabbit plasma extracts: (A) total-ion-chromatogram obtained by full scan GC–MS analysis, (B) selected-ion-monitoring of m/z 248 (TEB) and (C) selected-ion-monitoring of m/z 251 (IS-TEB). Arrows indicate retention time of TEB and IS-TEB.

blank plasma extract in the SIM (TEB, m/z 248; and IS-TEB, m/z 251) rather than the full-scan mode improved the selectivity in the retention region of TEB and IS-TEB but matrix interferences were still present near the IS-TEB peak and both near and at the retention time of the TEB peak (Fig. 3B and 3C). The SRM scheme used for the GC–MS–MS analysis resulted in a highly selective detection scheme for TEB in the rabbit plasma extract. The SRM mode completely eliminated the interferences throughout the entire retention time range of the analysis (Fig. 4).

3.3. Linearity

The GC–MS analysis of the TEB standards was linear from 1.7 to 270 ng. The low end of the GC–MS standard curve was biased by the presence of a co-eluting endogenous impurity that contributed to the integrated TEB peak area. The endogenous

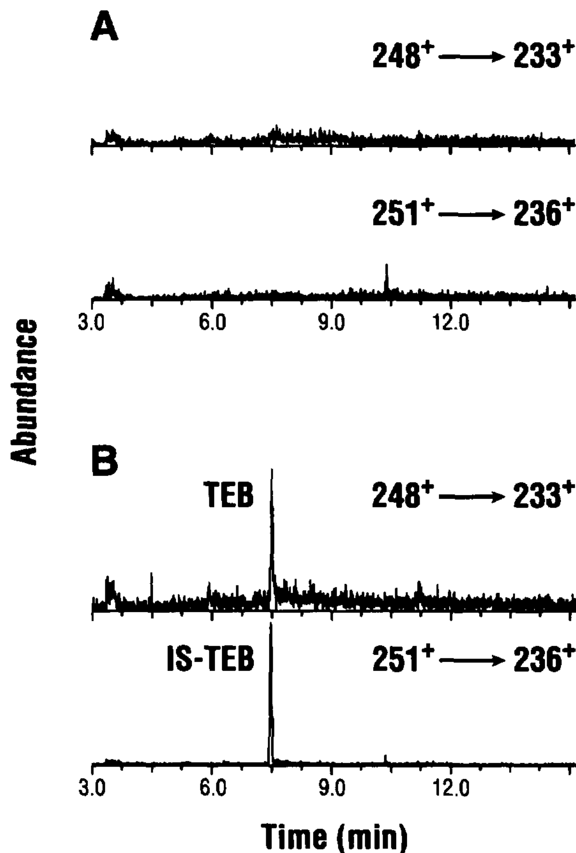

Fig. 4. Ion trap selected-reaction-monitoring (parent window m/z 247 to 255 and daughters m/z 233 (TEB) and m/z 236 (IS-TEB)) analysis of: (A) blank rabbit plasma extract and (B) 0.27 ng TEB plus 2.0 ng IS-TEB spiked rabbit plasma extract.

impurity in the blank plasma samples generated a signal equivalent to 0.26 ng of TEB. As a result, the first three standards were not used in the generation of the mean response factor. The response factors obtained for the GC–MS analysis of the remaining standards are shown in Table 1. The mean response factor was 2.10 with an R.S.D. value of 12.6%. Replicate injections (n=5) of the 2.7 and 27 ng standards resulted in R.S.D. values for the response factor of 6.2 and 2.0%, respectively. The GC–MS–MS analysis of the TEB standards was linear over at least three orders of magnitude (0.1 ng to 270 ng TEB with 2 ng IS-TEB), with a coefficient of determination of at least 0.999. The response factors obtained for each standard using the SRM scheme are shown in Table 1. The mean response factor for

Table 1
Tebufelone standard curve response factors (2.0 ng internal standard)

TEB mass (ng)	GC–MS–MS response factor	GC–MS response factor
0.11	1.44	ND[a]
0.27	1.36	ND
0.54	1.63	ND
1.1	1.72	1.76
2.7	1.71	1.92
5.4	1.69	2.01
11	1.75	2.03
27	1.75	1.94
54	1.74	2.31
110	1.74	2.29
270	1.70	2.56
Mean	1.66	2.10
S.D.	0.13	0.27
R.S.D.	7.8%	12.6%

[a]ND = not determined due to matrix interference.

the standards was 1.66 with an R.S.D. of 7.8%. Replicate injections (n=5) of the 2.7 and 27 ng standards resulted in R.S.D. values for the response factor of 3.3 and 1.0%, respectively.

3.4. Accuracy and precision

The accuracy and precision values obtained for the analysis of TEB spiked into rabbit plasma extracts at the 0.27, 2.7 and 27 ng level by GC–MS–MS and GC–MS are shown in Table 2. The matrix interferences observed with the SIM scheme prohibited the accurate analysis of TEB spiked at the 0.27 ng level. The percent of target for the 2.7 and 27 ng TEB

Table 2
Analysis of TEB spiked rabbit plasma extracts

TEB spike (ng)	TEB found (ng) Mean ± S.D.	Percent of target (Mean; R.S.D.)
A) *GC–MS–MS (SRM analysis)*		
0.27	0.32 ± 0.005	117%; 1.6%
2.71	2.64 ± 0.027	97%; 1.0%
27.1	24.9 ± 0.15	92%; 0.6%
B) *GC–MS (SIM analysis)*		
0.27	ND[a]	ND
2.71	3.04 ± 0.06	112%; 2.2%
27.1	28.9± 2.00	107%; 6.9%

[a] ND=not determined due to matrix interference.

spiked samples was 112 and 107%, respectively, with R.S.D. values of less than 7% for the GC–MS analysis. The superior selectivity provided by the SRM approach allowed the quantitation of TEB at all spiked levels. The percent of target obtained for the 0.27, 2.7 and 27 ng spiked samples were 117, 97 and 92%, respectively. The R.S.D. values for the replicate samples ($n=3$) at each level were less than 1.6%. The smaller %R.S.D. values obtained for the GC–MS–MS analysis, relative to the GC–MS approach, is due to the greater selectivity provided by the SRM scheme.

4. Conclusions

A benchtop ion trap GC–MS–MS operated in the SRM mode has been shown to provide superior selectivity, accuracy and precision, relative to a GC–MS operated in the SIM mode, for the analysis of a model drug compound in a plasma matrix. The current ion trap instrumentation overcomes the limitations of previous trap based instrumentation, allowing the generation of linear standard curves, over at least three orders of magnitude, based on stable-isotope-labeled internal standards. The SRM mode was demonstrated to eliminate interferences from coeluting matrix components encountered in the SIM mode for GC–MS analysis. The reasonable cost of the current benchtop ion trap GC–MS–MS instruments should open the possibility of increased selectivity and sensitivity via MS–MS analysis to a broader range of laboratories.

Acknowledgments

We gratefully acknowledge the technical advice and assistance of R.L.M. Dobson and R.J. Strife.

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