A Capillary Gas Chromatographic Procedure for the Analysis of Nine Common Residual Solvents in Water-Insoluble Bulk Pharmaceuticals

Q. Chan Li and **Kenneth A. Cohen***
Analytical Sciences Department, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877

G. Zhuang
State Key Laboratory of Estuary and Coastal Research, East China Normal University, Shanghai, P. R. China

Abstract

A direct-injection, split-mode capillary gas chromatographic procedure is developed for the analysis of nine solvents commonly used in the synthesis and purification of bulk pharmaceuticals. The nine solvents are methanol, methylene chloride, hexane, ethyl acetate, tetrahydrofuran, iso-octane, 1,4-dioxane, toluene, and dimethylformamide. The procedure is derived by modifying *U.S. Pharmacopeia* and *European Pharmacopeia* compendial methods. Modifications include raising injection temperature and revising temperature programming, leading to enhanced sensitivity and a shorter analysis time of 40 min.

For each solvent, the procedure is validated for selectivity, linearity, recovery, precision, quantitation limit, and detection limit. All nine solvents are completely resolved. Determination coefficients (r^2) are at least 0.998. Toluene has a linear response from 10 to 1000 ppm (with respect to a drug concentration of 100 mg/mL). All other solvents have linear responses from 20 to 2000 ppm. Recoveries range from 99.3 to 100.9%. Relative standard deviations for precision are not more than 2.8%. The quantitation limits (in ppm) are as follows: methanol, 8.6; methylene chloride, 95.3; hexane, 48.5; ethyl acetate, 23.5; tetrahydrofuran, 13.0; iso-octane, 24.0; 1,4-dioxane, 31.5; toluene, 10.0; and dimethylformamide, 14.9. Furthermore, a system suitability test is validated, and requirements are set. Finally, two drug substance samples are analyzed to show the suitability of the procedure, which can generally be used to determine any one or any combination of these nine residual solvents in water-insoluble bulk pharmaceuticals.

Introduction

Both U. S. Pharmacopeia 23 (USP 23) (1) and the International Conference on Harmonization (ICH) draft guidelines (2) require the determination of organic volatile impurities (referred to as "OVIs" in USP 23 and as "residual solvents" in ICH guidelines) as an essential element in the control of the quality of pharmaceutical products. Organic volatile impurities are often residual solvents that are used in synthesis and purification of drug substances but escape drying. In a recent review article (3), C. Witschi and E. Doelker discussed residual solvents in pharmaceutical products and addressed topics such as acceptable limits and analytical methods, among others. The authors reviewed analytical methods with emphasis on gas chromatography (GC). It is accepted that OVIs are most appropriately analyzed with GC. Direct (split or splitless) and headspace (dynamic or static) injections are common techniques of sample introduction in GC. Direct injection involves dissolving a drug in a suitable solvent and injecting this solution directly onto the column. This injection is rapid, convenient, and easily automated. The headspace technique introduces the vapors of OVIs onto the GC column. A major advantage of the headspace technique is the prevention of the introduction of nonvolatile materials onto the column, leading to an extended lifetime of the column. For water-soluble drugs, water is the dissolution medium of choice. For water-insoluble drugs, dissolution media are organic solvents.

Current official GC methods are described in USP 23 under chapter 467 (*Organic Volatile Impurities*) and in *European Pharmacopoeia* (Eur. Ph., V.3.3.9). Formerly there were six USP compendial GC methods, Methods I–VI (3–4). Methods II and III, which are based on dynamic headspace, were removed in 1993 and are no longer used to measure OVIs in pharmaceutical products. USP 23 describes four GC methods (Methods I, IV–VI) for the analysis of benzene, chloroform, 1,4-dioxane, methylene chloride, and trichloroethylene, and a method for methylene chloride in coated tablets. Methods I, V, and VI are based on direct injection. Method I is suitable for water-soluble drugs, and Method V is suitable for water-insoluble drugs. Method V was introduced primarily based on the work of Chen

* Author to whom correspondence should be addressed.

et al. (5). Method VI expands choices of columns and chromatographic conditions. Method IV uses the static headspace sampling technique and is limited to water-soluble drugs. To use the headspace technique for water-insoluble drugs, W. C. Kidd (6) and M. De Smet (7) suggested the use of organic-type dissolution media. Commonly used organic solvents are dimethylsulfoxide (DMSO), dimethylacetamide (DMA), and 1,3-dimethyl-2-imidazolidinone (DMI).

The EP specifies systems A and B for the analysis of seven solvents: acetonitrile, methylene chloride, chloroform, benzene, trichloroethylene, dioxane, and pyridine. The chromatographic conditions of system A correspond to those of USP 23 Method V, but if column contamination is a concern, headspace injection is used, as in Method IV of USP 23. System B, which uses a polyethylene glycol column (30 m × 0.32 mm × 0.53-mm i.d., 0.25-µm film thickness) is used if there are matrix interferences or solvent coelution.

In this paper, we describe the development and validation of a direct-injection, split-mode capillary GC procedure, modified from the USP and EP methods, to analyze a water-insoluble drug substance for nine common residual solvents: methanol (MeOH), methylene chloride, hexane, ethyl acetate, tetrahydrofuran (THF), iso-octane, 1,4-dioxane, toluene, and dimethylformamide (DMF). To our knowledge, the analysis of residual iso-octane has never been mentioned in any compendial method. We will demonstrate that our procedure can be used to determine any or all of the nine common residual solvents in water-insoluble bulk pharmaceuticals with sensitivity, accuracy, and simplicity.

Experimental

Reagents and materials

Solvents used in this method were suitable for GC and more than 99% pure, except that hexane as *n*-hexane was 89.95% pure. Methanol, methylene chloride, hexane, ethyl acetate, THF, and DMF were purchased from EM Sciences (Gibbstown, NJ). Iso-octane, 1,4-dioxane, and *n*-propanol were from Burdick & Jackson (Muskegon, MI). Toluene and DMSO were from J. T. Baker (Phillipsburg, NJ). Drug substance samples were obtained from the Chemical Process Department of Research and Development at Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT).

Chromatographic system and conditions

Experiments were performed on a Hewlett-Packard (HP) 6890 Series GC equipped with an HP GC autosampler controller and an HP 6890 series injector. Flame ionization detection (FID) was used. Chromatographic conditions were as follows. The column was an Rtx-1301, fused-silica, crossbound 6% cyanopropylphenyl–94% dimethyl polysiloxane (30 m × 0.53 mm, 3-µm film thickness, Restek, Bellefonte, PA).

The initial oven temperature of 45°C was held for 8 min and increased at 10°C/min to 195°C, at which temperature it was held for 17 min. The total run time was 40 min.

The injector temperature was 200°C. Injection was carried out in the split mode at a split ratio of 1:5, a split flow rate of 16.2 mL/min, and a total flow rate of 21.9 mL/min. The injection volume was 1 µL.

Helium was used as the carrier gas at a constant flow rate of 3.3 mL/min. Its velocity through the column was 25 cm/min at 45°C. The FID temperature was 260°C, and the FID flow rate was 30 mL/min hydrogen, 400 mL/min air. Helium was used as the makeup gas at a constant flow rate of 30 mL/min. The signal range was zero. Chromatographic data were collected and processed via a PE Nelson TurboChrom data management system (v. 4.1, Perkin-Elmer, Norwalk, CT).

Solution preparation

Internal standard stock solution

A 0.2-mL aliquot of *n*-propanol was pipetted into a 10-mL volumetric flask and diluted to volume with DMSO. Then 0.1 mL of this solution was pipetted into a 10-mL volumetric flask and diluted to volume with DMSO.

Diluent blank solution containing internal standard

A 1-mL aliquot of the internal standard stock solution was pipetted into a 10-mL volumetric flask and diluted to volume with DMSO. This solution was used to prepare standards and samples.

Standard stock solution

A 0.2-mL aliquot of each of the eight solvents (methanol, methylene chloride, hexane, ethyl acetate, THF, iso-octane, 1,4-dioxane, and DMF) and a 0.1-mL aliquot of toluene were pipetted into the same 10-mL volumetric flask and diluted to volume with DMSO. Then 0.1 mL of this solution was pipetted into a 10-mL volumetric flask and diluted to volume with DMSO.

Standard solution

A 1-mL aliquot of the internal standard stock solution and 1 mL of the standard stock solution were pipetted into the same 10-mL volumetric flask and diluted to volume with DMSO. This solution was also used for the system suitability test.

Sample preparation

A drug substance poorly soluble in water was dissolved with a concentration in the range of 20–100 mg/mL in the diluent blank solution containing the internal standard.

Quantitation

The concentration (ppm) of the residual solvents in the drug substance sample was calculated by using a combination of the internal (*n*-propanol) and external standards. The equation is as follows:

$$\text{ppm of solvent } i = (r_{i,\mu}/r_{i,s}) \times (c_{i,s}/c_{\mu}) \times 10^6$$

where $r_{i,\mu}$ is the ratio of area response of solvent i to the internal standard in a sample injection (note that the area response of solvent i is adjusted from blank interference, if applicable), $r_{i,s}$ is the average area response ratio of solvent i to

the internal standard from six standard injections (assuming $r_{i,s}$ is 1 for unknown peaks), $c_{i,s}$ is the concentration (mg/mL) of solvent i in the standard solution, and c_{μ} is the concentration (mg/mL) of the drug substance sample.

Results and Discussion

Method development

Initially we tried USP Method IV using DMSO and DMA (dimethylacetamide) as dissolution media and found that impurities in these solvents were enriched in headspace and interfered with the analysis of some residual solvents (e.g., methylene chloride, methanol, and DMF).

Next we tried USP Method V using DMSO as the dissolution medium and encountered two problems. First, early eluting peaks including methanol, methylene chloride, and hexane were broad. Second, the sensitivity of DMF was low due to both its high boiling point (153°C) and low injection temperature (140°C, as specified in Method V). We modified the method by using split injection as prescribed in System A in the EP and raising the injector temperature to 200°C. The split injection produced narrower peaks, especially for those early eluting solvents cited above, and thus enhanced sensitivity. The injection temperature of 200°C ensured the complete vaporization of DMF and therefore increased its sensitivity. It should be noted that an injection temperature of 200°C is generally applicable, as most drug substances are thermally stable at this temperature. However, a lower injection temperature may be needed if a drug substance is not stable at 200°C. We also revised temperature programming to complete an analysis in 40 min. Finally, *n*-propanol was used as the internal standard in conjunction with external standards for quantitation.

Method validation

Selectivity

The selectivity of this procedure is demonstrated in Figures 1 and 2. Figure 1 is a representative chromatogram of the DMSO blank with the internal standard. Figure 2 shows a representative standard chromatogram that indicates complete separation of all nine solvents and the internal standard. Comparison of Figures 1 and 2 shows that the blank posed no interference with any of the solvents. In addition, the DMSO blank without the internal standard was injected (data not shown) and showed no interference with the internal standard.

Linearity

The linearity of the area response of each solvent was determined at concentrations ranging from approximately 20 to 2000 ppm (from approximately 10 to 1000 ppm for toluene). Each concentration level was injected in duplicate. Linear regression data are presented in Table I. Within the specified ranges, each determination coefficient (r^2) was at least 0.998. Therefore, each solvent had a linear response.

Recovery

Recovery for each of the nine solvents was validated by spiking a drug sample preparation with approximately 100 ppm of standard toluene and approximately 200 ppm each of standard methanol, methylene chloride, hexane, ethyl acetate, THF, iso-octane, 1,4-dioxane, and DMF. The spiked sample was injected in three replicates as was a drug substance sample that was not spiked. For a given solvent, if there was interference from the unspiked sample, the peak area of the unspiked sample injection was subtracted from that of the spiked sample injection. Then the recovery of each solvent was calculated by comparing a recovered concentration with a spiked, known concentration. Results are summarized in Table II. Average recoveries ranged from 99.3 to 100.9%; none of the relative standard deviations (RSDs) were more than 2.3%, indicating acceptable recovery for all nine solvents.

Precision

Precision was validated by injecting in duplicate each of five separate preparations of the standard solution. For each solvent, the average area response and an RSD were calculated from the 10 injections. Results are summarized in Table III. None of the RSDs were more than 2.8%, indicating acceptable precision.

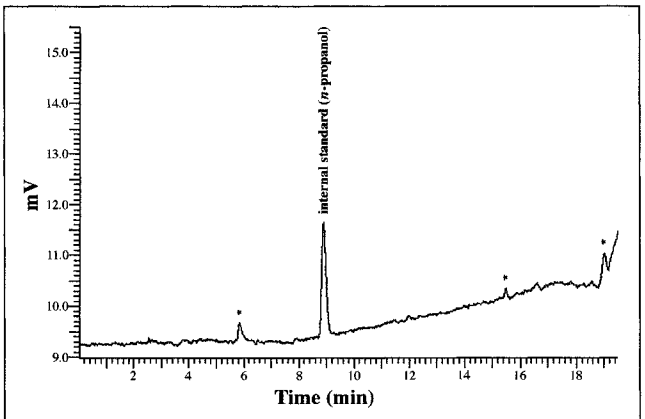

Figure 1. Chromatogram of the DMSO blank containing the internal standard. Asterisks (*) represent impurities in the DMSO blank. DMSO eluted at approximately 20.3 min.

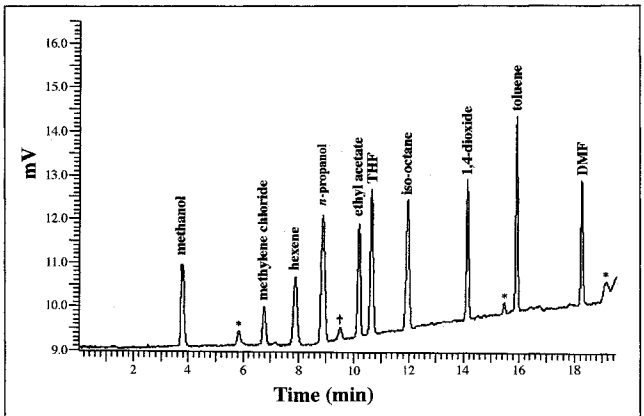

Figure 2. Chromatogram of a standard/system suitability injection. Asterisks (*) represent impurities from the DMSO blank. The dagger (†) represents impurity from hexane. Approximate retention times (min): methanol, 3.76; methylene chloride, 6.78; hexane, 7.89; *n*-propanol, 8.89; ethyl acetate, 10.21; THF, 10.66; iso-octane, 11.97; 1,4-dioxane, 14.15; toluene, 15.93; DMF, 18.25; DMSO (not shown), approximately 20.3.

Table I. Linearity: Response Versus Concentration

Component	Concentration (ppm)		Response area Injection 1		Response area Injection 2
Methanol	16		1777		1951
	32		2697		3364
	40		2627		3136
	63		6021		6025
	79		6342		6469
	158		13831		13319
	395		32514		32780
	633		54223		54156
	791		65773		66102
	1582		132740		131791
		Slope	83.5	r^2	0.9998*
		y-Intercept	233.4		
Methylene chloride	53		1436		1169
	66		1716		1946
	106		2385		2717
	132		3036		2893
	265		6707		6413
	662		14892		14917
	1060		27069		26682
	1325		32888		32589
	2650		64670		64066
		Slope	24.2	r^2	0.9991*
		y-Intercept	–20.6		
Hexane	24		1676		1793
	30		2015		2053
	48		3595		4222
	60		4331		4006
	121		8234		8426
	302		25186		25218
	484		42568		41268
	604		51337		51412
	1209		97882		97682
		Slope	82.3	r^2	0.998*
		y-Intercept	–85.4		
Ethyl acetate	18		1752		1968
	36		3184		3306
	45		3030		3386
	72		5641		6489
	90		7375		7308
	180		16105		16255
	451		37865		38170
	722		66928		66558
	902		81772		81582
	1804		161558		161700
		Slope	90.1	r^2	0.9995*
		y-Intercept	–342.2		
Tetrahydrofuran	18		2242		1564
	36		3826		3796
	44		4186		4376
	71		8746		8432
	89		9294		9113
	178		21492		20416
	444		47223		47245
	711		85325		84893
	889		103928		103874
	1778		204932		204599
		Slope	115.9	r^2	0.9995*
		y-Intercept	–379.0		
Iso-octane	14		721		997
	27		2097		2414
	34		2852		3047
	55		5915		5931
	69		5843		6117
	137		15278		15151
	343		37295		37736
	548		64711		64705
	685		79191		78688
	1370		161265		161812
		Slope	118.5	r^2	0.9997*
		y-Intercept	–1312.1		
1,4-Dioxane	21		1453		1345
	41		3417		3424
	52		3811		3981
	83		6285		6515
	103		6952		7292
	207		16206		16349
	516		37640		38215
	826		66022		64928
	1033		80081		80205
	2066		160467		159787
		Slope	77.6	r^2	0.9997*
		y-Intercept	–243.8		
Toluene	9		2033		2079
	17		3601		4306
	22		4399		4599
	34		8093		7765
	43		9018		8922
	86		20277		19445
	216		45441		46191
	345		80797		81036
	431		98148		97775
	862		195150		195050
		Slope	227.1	r^2	0.99995*
		y-Intercept	–190.4		
Dimethyl formamide	19		1171		1494
	38		2007		2770
	47		2557		3116
	76		5499		5412
	94		6375		6327
	189		12975		13728
	472		31232		31541
	755		54727		56065
	944		67168		67264
	1888		133414		132330
		Slope	70.8	r^2	0.9994*
		y-Intercept	–154.4		

* Determination coefficient (r^2) was given to the first digit that was not nine.

Detection and quantitation limits

The limits of detection (LOD) and quantitation (LOQ) for each of the nine solvents were determined according to the following equation:

$$\text{LOQ or LOD} = K\,(S_B/S)$$

where K equals 3 for LOD or 10 for LOQ, S_B is the standard deviation of the peak area response at a concentration typically yielding a peak signal-to-noise ratio of 5–20, and S is the sensitivity of a solvent (area/weight). LODs and LOQs were determined relative to a drug concentration of 100 mg/mL. The results given in Table IV indicate that the method is sensitive.

System suitability test

A system suitability test was developed to monitor the overall GC system, including analytical column performance. The standard solution was used as the system suitability test solution. Because ethyl acetate and THF were the most closely eluted pair, this critical pair was chosen to set test requirements. The requirements of this test were met when the resolution between ethyl acetate and THF was at least 1.9. This

Table II. Recovery (%) for Nine Solvents

Injection	Methanol	Methylene chloride	Hexane	Ethyl acetate	THF	Iso-octane	1,4-Dioxane	Toluene	DMF
1	97.4	100.4	100.5	101.1	100.2	100.0	98.6	100.5	100.4
2	100.2	99.3	103.4	99.5	99.4	99.8	100.6	100.0	100.5
3	100.2	98.2	98.9	100.4	102.4	100.5	100.5	100.1	98.6
Average	99.3	99.3	100.9	100.3	100.7	100.1	99.9	100.2	99.8
RSD (%)	1.6	1.1	2.3	0.8	1.5	0.4	1.1	0.3	1.1

Table III. Precision

	Area response									
Injection	Methanol	Methylene chloride	Hexane	Ethyl acetate	THF	Iso-octane	1,4-Dioxane	Toluene	DMF	*n*-Propanol
1-1	14381	6550	13802	17104	21916	22008	16286	20398	13339	25019
1-2	14865	6823	14436	16795	22127	22398	16790	20364	13581	25085
2-1	14246	6582	14236	17332	21919	21569	16435	20503	13415	25199
2-2	14526	6682	13913	16661	22124	21646	16286	19976	13886	24160
3-1	14374	6878	14456	16535	21810	21607	16377	20632	13529	25081
3-2	14053	6328	13139	16868	21557	21629	15939	20436	13321	25160
4-1	14670	6553	13893	16884	21349	20976	16596	20186	13607	24929
4-2	14395	6786	14311	17077	22336	22258	16779	20631	13765	24537
5-1	14088	6872	14290	16736	21528	21700	16650	20041	13656	25164
5-2	14522	6657	14009	16899	21235	22172	16432	20782	13528	24226
Average	14412	6671	14048	16888	21790	21796	16457	20395	13563	24856
RSD (%)	1.7	2.6	2.8	1.4	1.7	1.9	1.6	1.6	1.3	1.6

Table IV. Detection Limits and Quantitation Limits (ppm)

	Methanol	Methylene chloride	Hexane	Ethyl acetate	THF	Iso-octane	1,4-Dioxane	Toluene	DMF
Detection limit	2.6	28.6	14.5	7.0	3.9	7.2	9.5	3.0	4.5
Quantitation limit	8.6	95.3	48.5	23.5	13.0	24.0	31.5	10.0	14.9

Table V. System Suitability Precision

		Injection						
Compound	Parameter	1-1	1-2	1-3	2-1	2-2	2-3	RSD (%)
Ethyl acetate	Retention time (min)	10.21	10.21	10.21	10.21	10.21	10.21	0.0
	Area	17104	16795	17322	16661	16535	16868	1.7
THF	Retention time (min)	10.66	10.66	10.66	10.66	10.66	10.66	0.0
	Area	21916	22127	21919	22124	21810	21557	1.0
Ethyl acetate/THF	R_s	2.12	2.39	2.46	2.14	2.21	2.26	6.0

Table VI. Residual Solvent Contents (ppm) in Drug Substance Samples

Solvent	Batch 1	Batch 2
Methanol	undetected	undetected
Methylene chloride	undetected	undetected
Hexane	382	1040
Ethyl acetate	219	197
Tetrahydrofuran	undetected	25
Iso-octane	undetected	undetected
1,4-Dioxane	undetected	undetected
Toluene	undetected	16
Dimethylformamide	undetected	undetected
Unknown #1	11	33
Unknown #2	38	109
Unknown #3	undetected	26

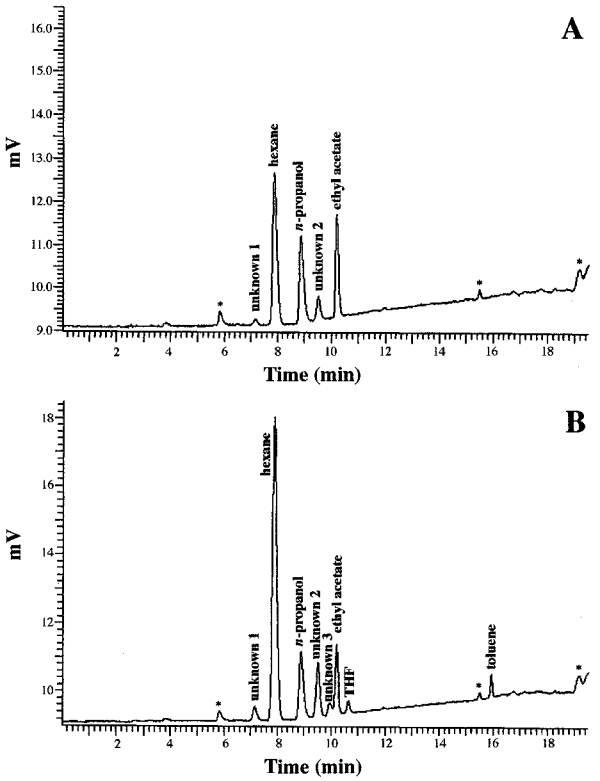

Figure 3. Representative chromatograms of sample injections. (A) Batch 1. (B) Batch 2. Asterisks (*) represent impurities in the DMSO blank. DMSO eluted at approximately 20.3 min.

minimum resolution ensured complete separation of ethyl acetate and THF as well as all other residual solvents.

System suitability precision was measured by injecting in triplicate each of two test solutions onto an equilibrated GC system. For ethyl acetate and THF, RSDs (six replicates) for retention time (RT) and area response were calculated. Resolution (R_s) between ethyl acetate and THF was also calculated. Each parameter had an RSD not more than 6.0%, and the replicate injections were therefore deemed precise. Results are summarized in Table V.

Sample analysis

For the purpose of verification, two drug substance batches whose synthesis and purification involved the use of these nine solvents were analyzed according to this procedure. Three sample preparations for each batch and one injection per preparation were made. The average results for each batch are shown in Table VI. Figure 3 shows a representative chromatogram for each batch. Although not all nine solvents were present and a few unknown volatiles were detected, the suitability of the method was clearly demonstrated.

Conclusion

A direct-injection, split-mode capillary GC procedure was developed for the analysis of nine common residual solvents in water-insoluble drugs. To our knowledge, it is the first reported method for iso-octane. This procedure was validated to be selective, sensitive, linear, accurate, and precise in the range of interest. It has been shown that the procedure can generally be used to determine any one or any combination of these nine residual solvents in water-insoluble bulk pharmaceuticals.

Acknowledgment

We thank Roxane Lee of Boehringer Ingelheim Pharmaceuticals, Inc. for reviewing this manuscript.

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Manuscript accepted December 5, 1997.