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GAS CHROMATOGRAPHIC DETERMINATION OF RESIDUAL LEVELS OF METHANOL AND CHLOROFORM FROM LIPOSOMAL, MICROSPHERES AND NANOPARTICLES

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ABSTRACT

The use of liposomal formulations has rapidly gained popularity in pharmaceutical research and development. Their preparation often involves the use of organic solvents such as methanol and chloroform to dissolve lipophilic lipids. In the present study, gas chromatographic method for the determination of methanol and chloroform residual levels in liposomes was developed using GC 17 A Shimadzu with FID (a flame ionization detector) and the separation was carried out on BP 624 column (4% cyanopropyl phenyl and 94% dimethyl silixone, 30 m X 0.53 mm i.d. X 0.25 μm coating thickness), with nitrogen as a carrier gas in the split mode by direct injection method. The method was validated according to ICH guidelines. The method described is simple, sensitive, rugged, reliable and reproducible and requires less time than other reported methods for the quantitation of methanol and chloroform levels from liposomal formulations of lamivudine and stavudine.

Key words: Lamivudine, Stavudine, Residual solvents, Gas chromatography, Methotrexate, Ketorolactromethamine, Ketoprofen, Nanoparticles, Serapeptase.

INTRODUCTION

Liposomes[1] are lipid vesicles used as drug carriers. The use of liposomal formulations is rapidly gaining popularity in pharmaceutical research and development. Their preparation often involves the use of organic solvents such as chloroform, methanol, diethyl ether, *tert.*-butanol etc. to dissolve the phospholipids. But official analytical monographs include a test for volatile impurities (residual solvents)[2]. Thus, determination of residual solvents in the liposomal formulation is also an important evaluation, in order to conclude whether the prepared formulation contains the harmful solvents within the ICH

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limits. The commonly used technique for such estimation is gas chromatograph[3-6]. The method described is simple, sensitive, rugged, reliable and reproducible and requires less time.

Globalization and manufacture of the formulation for global market has necessitated conformance of such organic volatile impurities to limits prescribed by ICH guidelines. For the said purpose, methods have to be developed and validated for their detection and quantification. Although USP[7] has prescribed certain methods for their estimation, the emergence of a plethora of technological innovations has thrown challenges to the analyst to learn and apply the instruments for productive emergence of data on the presence of organic volatile impurity in marketed formulation. This project has been selected keeping in view the importance of the residual solvent testing in drug formulations especially for herbal origin.

Organic volatile impurities[8-10] have to be tested to check whether they are with in the specified ICH limits. Documentation of the data pertaining to the actual organic solvent impurity levels is not a compulsion over a strip of formulation. It is the moral responsibility of pharmaceutical manufacturers to control the organic volatile impurity level in the formulation below the permissible limits as they posses a potential risk to human health and also play a vital role in regulating the physicochemical properties of the bulk drug substances.

EXPERIMENTAL

Gas chromatographic conditions

Objective

To develop a suitable method for the simultaneous estimation of methanol, and chloroform and apply the same for their quantification in the liposomal formulation.

Equipment

A gas chromatograph GC-17A Version-3 equipped with flame ionization detector (FID) was used.

In the proposed method, the following working instrumental variables were enabled on the gas chromatograph. The column temperature was maintained at 200^0C, injection port temperature was 230°C and detector temperature was 250°C. The flow rate of carrier gas was 2 mL/min with control mode of split of ratio 1 : 22. The column pressure

was initially maintained at 19 kp**a.**

Chemicals required

Methanol, chloroform and dimethyl sulfoxide All the reagent were of analytical grade.

Preparation of standard

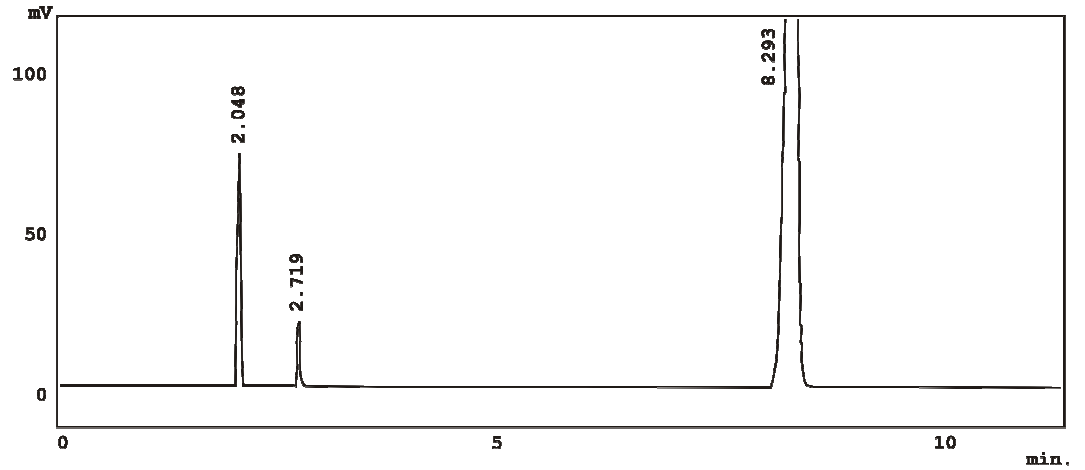
Dimethyl sulphoxide (DMSO) was selected as the standard and sample diluent, based on its ability to dissolve wide variety of substances. Also DMSO is a solvent with high boiling point that does not interfere with more volatile solvents tested by GC for the method involving analysis of high boiling point solvents. Standard stock of methanol and isopropyl alcohol were prepared by diluting with DMSO in 10 mL volumetric flask to get concentration of 1000μLmL^{-1}. From these stocks 8 serial working standard solutions were prepared to obtain concentrations ranging from 10-4000 ppmmL^{-1} and 1-240 μLmL^{-1} for methanol and chloroform, respectively. The volume is made with DMSO. 1 μL of working standards were injected into gas chromatograph and standard calibration curves were obtained for methanol and isopropyl alcohol.

Preparation of sample

Accurately weighed 1 g sample dissolved and sonicated with DMSO, filtered through Whatman filter paper No 1 and volume was made up to 10 mL with DMSO, in separate 10mL volumetric flask. From these samples, 1 μL samples were injected and concentrations of methanol and chlolroform in sample were calculated by interpolating standard calibration curve.

Analytical method validation

Analytical method validation is the process of demonstrating that analytical procedures are suitable for their intended use, more specifically. Analytical method validation is a matter of establishing documented evidence that provides a high degree of assurance that the specified method will consistently provide accurate test results that evaluate a product against its defined specifications and quality attributes. Method validation is also a holistic process that requires suitable instrumentation and competence in laboratory techniques to ensure success.



S.No.	Time	Area	Height	Conc.
1	2.048	135618	73936	6.0431
2	2.719	46018	20205	2.0502
3	6.293	2062905	233196	91.9076
		2244541	327296	100.0000

Fig. 1: Standard chromatogram for methanol and chloroform

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

Procedure: In a 10 mL volumetric flask 0.1 mL of methanol was taken and the volume was made upto 10 mL with dimethyl sulphoxide.(first dilution 100 ppm/µL). Further 0.1 mL was diluted to 10 with dimethyl sulphoxide (100ppb/ µL). Then volumes of 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1µL were injected into gas chromatograph. The detection limit was determined by the injected amount that results in a peak with a height at least twice or three times as high as the baseline noise level and the limit of detection was found to be 100 ppb.

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range.

Procedure:

In a series of 8 volumetric flasks of 10 mL volume, 0.1, 0.2, 0.4, 0.8, 1, 1.6, 3.2 and 4 mL of methanol were taken and the volume made up to 10 mL with dimethyl sulphoxide (100-4000 ppm/µL). Then volume of 1 µL was injected into gas chromatograph. The linearity is determined by a series of 3 injections of standards.

Table 1. Linearity data of methanol

Conc.	Av. area	SD	RDS
100	94247	457.6726	0.485606
200	195361	505.0947	0.258544
400	499587	540.5503	0.101899
800	1569255	22984.02	1.464645
1600	3155004	7240.781	0.229502
3200	6364859	24687.19	1.276085
4000	7774941	44507.75	0.317522

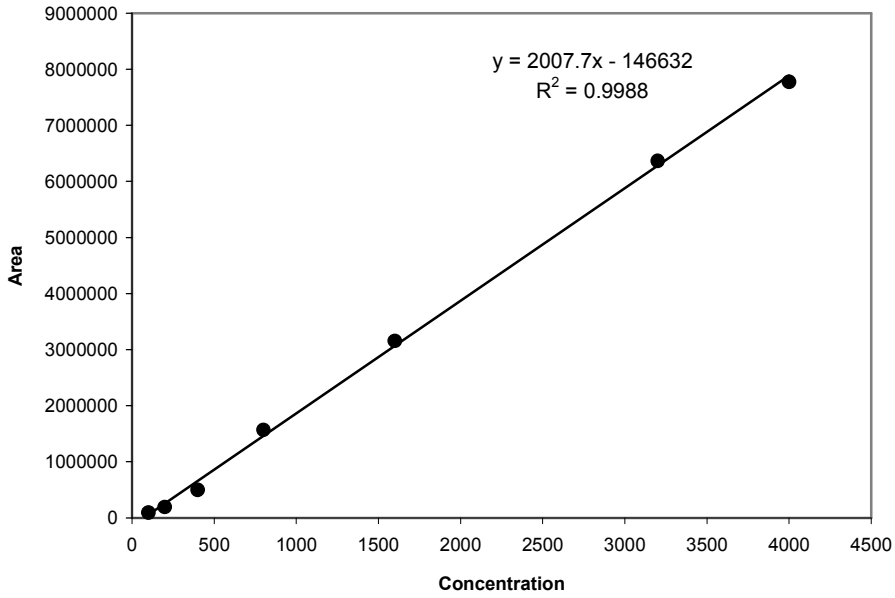


Fig. 2: Linearity of methanol

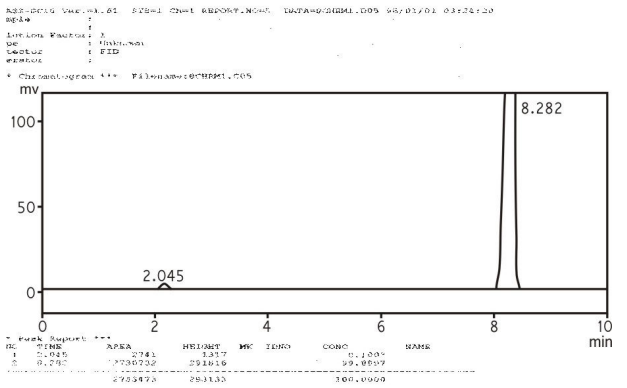


Fig. 3: Chromatogram of blank Lamivudine

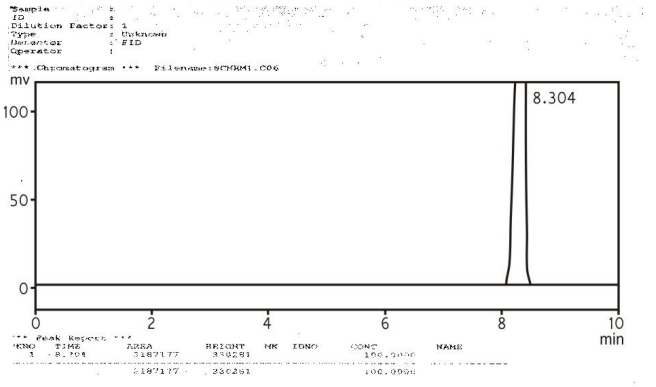


Fig. 4: Chromatogram of Lami formulation

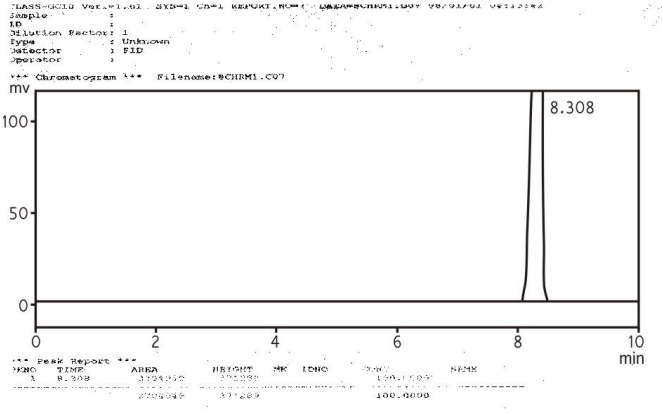
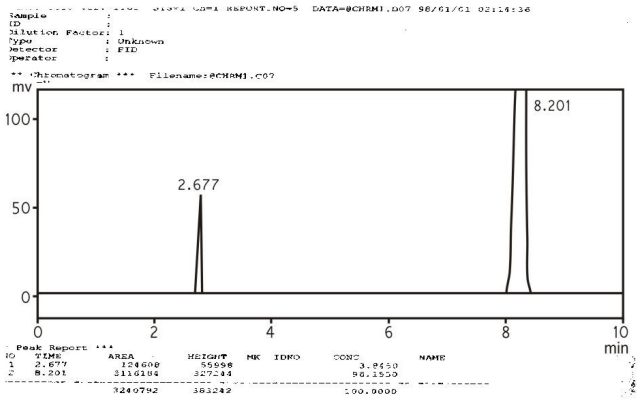
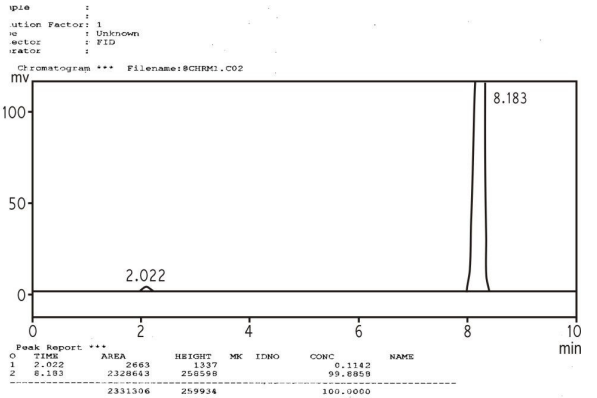


Fig. 5: Chromatogram of Stavi formulation



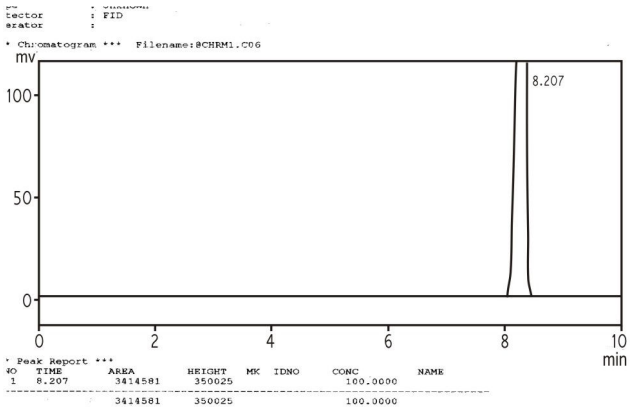
Peak Report ***						
NO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2.677	124608	55598			3.8650	
2	8.201	3116184	327244			96.1350	
		3240792	383242			100.0000	

Fig. 6: Chromatogram of standard chloroform



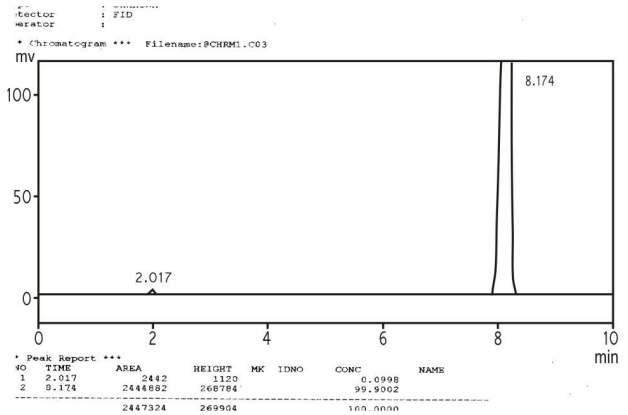
Peak Report ***						
NO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2.022	2663	1337			0.1142	
2	8.183	2328643	258598			99.8858	
		2331306	259934			100.0000	

Fig. 7: Chromatogram of sample 1 - blank



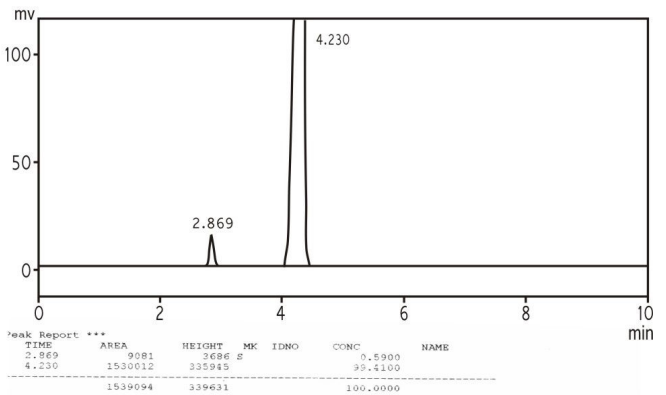
Peak Report ***						
NO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	8.207	3414581	350025			100.0000	
		3414581	350025			100.0000	

Fig. 8: Chromatogram of sample 2-liposome containing sodium cholate



NO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2.017	2442	1120			0.0998	
2	8.174	2444882	268784			99.9002	
		2447324	269904			100.0000	

Fig. 9: Liposome containing sodium cholate



TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
2.869	9081	3686	S		0.5900	
4.230	1530012	335945			99.4100	
	1539094	339631			100.0000	

Fig. 10: Chromatogram of KTAL

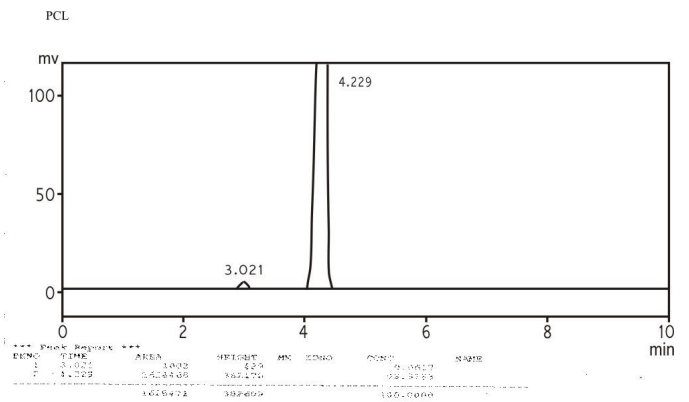


Fig. 11: Chromatogram of sample 5 PCL methanol

The response should be directly or by means of a well defined mathematical calculation proportional to the concentration of the analytes. The evaluation is made by visual inspection of a plot of signal height or peak area as a function of analyte concentration. The data of peak areas as obtained from the chromatograms for methanol (Fig. 1) are presented in table and the corresponding linearity curves are plotted as presented in Fig. 2.

System suitability parameters

Linearity was found in the range of 100-4000 ppm. HETP is 6.366 and the number of theoretical plates per meter is 4712.5 and the resolution was found to be 0.1333.

Formulation 1

Residual solvent analysis of liposomal formulation of lamivudine and stavudine

Trial 1: This was the analysis of blank formulation. 0.1 mL of the liposomal blank formulation was mixed with DMSO and made up to 10 mL with DMSO to extract the residual solvents. The solution was filtered through Whatman No. 1 filter paper. 0.1 mL of the filtrate was made up to 10 mL with DMSO.1 µL of this was injected into the gas chromatograph and the chromatogram was obtained.

Trial 2: The lamivudine liposomal formulation was analyzed. 0.1 mL of the liposomal formulation was mixed with DMSO and made up to 10 mL with DMSO to extract the residual solvents. The solution was filtered through What man No 1 filter paper. 0.1 ml of the filtrate was made up to 10 mL with DMSO. 1 µL of this was injected into the gas chromatograph and the chromatogram was obtained.

Trial 3: The stavudine liposomal formulation was analyzed. 0.1 mL of the liposomal formulation was mixed with DMSO and made up to 10ml with DMSO to extract the residual solvents. The solution was filtered through Whatman No. 1 filter paper. 0.1 mL of the filtrate was made up to 10 mL with DMSO. 1 µL of this was injected into the gas chromatograph and the chromatogram was obtained.

Formulation 2

Residual solvent Analysis of liposomes of methotrexate in the treatment of psoraisis

To detect the amount of residual solvent from linearity graphy by interation method for the samples mentioned below.

Sample 1 Blank

Sample 2 Liposome containing sodium cholate

Sample 3 Liposome containing sodium dodecyl sulphate

Sample 4 Liposome containing sodium deoxy cholate

Further the best liposmal formulation was incorporated onto a gel base for carrying out pre-clinical studies.

Sample 5 Methosome gel drug methotrexate

Formulation 3

Residual solvent analysis in microspheres Ketorolactromethamine loaded bovine serum albumin microspheres (KTAL), Ketoprofen loaded bovine serum albumin microspheres (KPAL)

Sample : 1 KTAL

Sample : 2 KPAL

Formulation 4

Residual solvent analysis of liposomes and nanoparticles of serapeptase

i. Drug loaded liposome formulation (soy pc- drug liposome)

ii. DMPC- drug liposome

iii. DMPE - drug liposome

iv .Chitosan nanoparticles

v. PCL nanoparticles.

Table 2. Amount of methanol was determined by interpolation only in these formulations

In house formulations	Solvent	Area	Concentration (ppm)

Blank Lamivudine	Methanol	2741	1.7
Liposome blank	Methanol	2663	1.5
Liposome containing sodium cholate	Methanol	2442	1.38
KPAL	Methanol	9081	5.14
PCL	Methanol	1003	0.56

CONCLUSION

The amounts of residual solvent present in the in-house formulations are with in the limits of the ICH guidelines, which in 3000 ppm for methanol.

REFERENCES

1. V. G. Prasad, A. C. Peter, and B. M. Ram. Gas Chromatographic Determination of Residual Levels of *tert.* -butanol from Lyophilized Liposomal Formulations, J Chrom. Biomed. Appli, **620(1)**, 83-88 (1993).
2. C. R. Lee, N. V. Dau and M. Krstulovic. Artefact Formation in the Determination of Residual Solvents According to a Method of the European Pharmacopeia, Int. J. Pharm., **195**, 159–169 (2000).
3. S. A. Coran, V Giannellini, S. Furlanetto, M. B. Alberti and S. Pinzauti Improving Gas Chromatographic Determination of Residual Solvents in Pharmaceuticals by the Combined Use of Headspace Solid-phase Microextraction and Isotopic Dilution, J Chrom A, **915**, 209–216 (2001).
4. Y Liu and C. O. Hua. Establishment of a Knowledge Base for Identification of Residual Solvents in Pharmaceuticals. Anal, Chem. Acta. **575**, 246–254 5 (2006).
5. B. Clayton, Hymer. Residual Solvent Testing, A Review of Gas Chromatographic and Alternative Techniques. Pharm, Res **23**, 337-344 (2003).
6. J. E. Haky, T. M. Stickney. Automated Gas Chromatographic Method for the Determination of Residual Solvents in Bulk Pharmaceuticals. J Chromatogr, A **321**,137-144 (1985).
7. United States Pharmacopeia, (USP-NF XXIV), Rockville, Maryland, United States Pharmacopoeial Convention Inc., (1995), 1877.
8. M. A. Karen, Handbook of Modern Pharmaceutical Analysis. Vol. III, Separations science technology, Academic Press, (2001) pp. 86-95.

9. C. Costin, Camarasu . Head Space SPME Method Development for the Analysis of Volatile Polar Residual Solvents by GC-MS, J. Pharm Biomed Anal. **23**,197-210 (2000).

10. Z Osawa, M. Aiba. Effect of Residual Solvent on Photodegradation of Poly (Vinyl Chloride),. Poly. Photochem **2**, 339-348 (1982).

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