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AUTOMATED GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RESIDUAL SOLVENTS IN BULK PHARMACEUTICALS

JEROME E. HAKY* and THOMAS M. STICKNEY
Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105 (U.S.A.)
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SUMMARY

A generalized gas chromatographic method is described for the determination of a wide variety of residual solvents and other volatile impurities in bulk pharmaceuticals. The method employs a highly selective graphitized carbon-black stationary phase, which is demonstrated to retain compounds on the basis of a linear combination of their boiling points and molecular volumes (*i.e.*, molecular weight divided by density). An autosampler is utilized to optimize injection precision and to provide for high sample throughput. Analytical data from replicate determinations of seven representative compounds are reported, and it is shown that calibration of the chromatographic systems against external standards produces comparable results to those obtained by standard addition techniques.

INTRODUCTION

Quantitating the amount of residual solvents and other volatile contaminants in bulk pharmaceuticals has become an important analytical problem, owing to the increasingly strict regulations on the subject. While the problem is normally addressed by the development of methods specific for the type of drug and residual solvent being analyzed, a generalized method for a variety of drugs and solvents is clearly advantageous.

Although a few generalized methods have been reported for the determination of residual solvents in pharmaceuticals, these have been based on either dynamic[1] or static[2] head-space gas chromatographic (GC) techniques, which require specialized equipment and are not easily adaptable to commercial autosampling units. Simpler methods not requiring headspace equipment have been developed for the determination of trace solvents in other matrices, including fermentation broths[3], film-coated tablets[4,5] and water[6].

The goal of the present study is the development of a general residual solvent method requiring only standard gas chromatographic equipment and lending itself to automation. In this report, we describe such a method, as well as a statistical analysis of the precision and accuracy of residual solvent determinations obtained on some representative bulk drugs.

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EXPERIMENTAL

Materials

Authentic samples of each potential residual solvent were obtained from Aldrich (Milwaukee, WI, U.S.A.). Experimental and bulk samples of pharmaceutical compounds were synthesized in these laboratories.

Apparatus and conditions

All (GC) analyses were performed on a Varian Model 6000 gas chromatograph equipped with a flame ionization detector, a Varian Model 8000 autosampler and a 6 in. × 2 mm I.D. glass column. The first 2 in. of the column (injector end) were packed with 3% OV-101 on 100–110 mesh Anachrom Q (Analabs, North Haven, CT, U.S.A.). The rest of the column was packed with 3% SP1500 on 80–120 mesh Carbopack B (Supelco, Bellefonte, PA, U.S.A.). Chromatograms were recorded and processed on a Varian Model 402 data system. Carrier gas (nitrogen) flow-rate was set at 20 ml/min, while hydrogen and air flow-rates were 30 and 350 ml/min, respectively. Injector and detector oven temperatures were set at 300°C and 325°C, respectively.

The GC determination of all residual solvents was obtained using a single four-step oven temperature program (Table I), which successfully resolved 13 of 15 common solvents. A simpler three-step program, also described in Table I, was later found to give nearly equivalent results (see chromatogram, Fig. 1).

Procedure

Samples to be determined were prepared by dissolution in benzyl alcohol at levels giving expected residual solvent concentrations in the range of 0.002–0.100 mg/ml. For a sample containing up to 1% (by weight) residual solvent, this corresponds to the preparation of a solution of the drug at a concentration of 10 mg/ml.

Analyses of the solutions were performed by injecting 7 μl onto the chromatographic system described above, using the autosampler. Peak heights corresponding to each relevant solvent were measured, and the concentrations of each solvent were calculated from a calibration curve which had been prepared from peak height data obtained from the chromatograms of solutions of each solvent in benzyl alcohol over

TABLE I

COLUMN OVEN TEMPERATURE PROGRAMS

Program	*Step*	*Initial temperature (°C)*	*Final temperature (°C)*	*Rate (°C/min)*	*Hold time (min)*
1	1	40	40	0	4
	2	40	90	2.5	0
	3	90	130	10	10
	4	130	220	30	26
2	1	40	40	0	4
	2	40	150	5	10
	3	150	220	35	23

a concentration range of 0.002–0.1 mg/ml. Calibration curves for each of the 15 solvents shown in Fig. 1 were found to be linear over this range (correlation coefficients >0.99).

The precision of the analytical system was determined through six replicate injections of solutions of each potential residual solvent in benzyl alcohol. Results are shown in Table II. The precision of the entire analytical method was measured through six replicate determinations of solvent content on each of seven different drugs. Results are summarized in Table III.

To check the accuracy of the method, residual solvent determinations on each sample were also made by the method of standard additions. Known amounts of each residual solvent were successively added to each of the sample solutions, over a concentration range of 0.002–0.1 mg/ml. Peak height data from the chromatograms of the original and spiked solutions were plotted linearly against the added concentrations, and the concentration of the residual solvent in the sample solution was determined by extrapolating a line to the abscissa. Results are summarized in Table III. Standard deviations and confidence intervals for these single determinations were estimated by the method described by Larsen *et al.*[7].

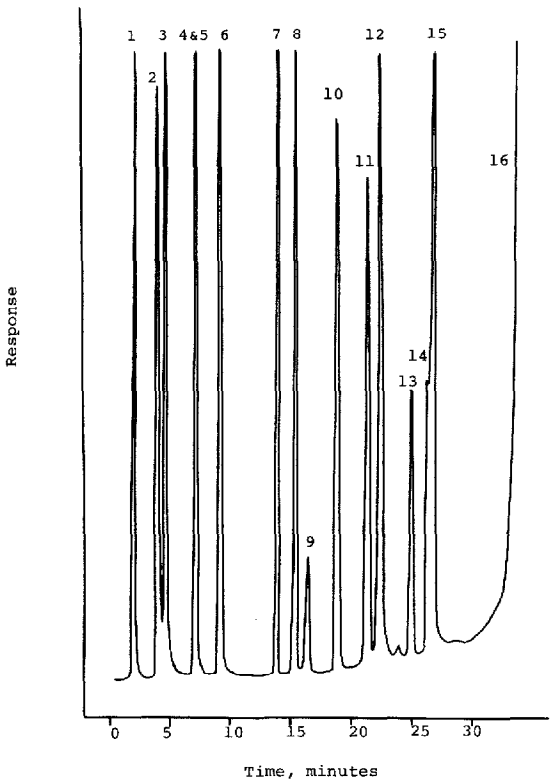

Fig. 1. Separation of 15 common residual solvents using temperature program number 2. Peaks: 1 = methanol; 2 = acetonitrile; 3 = ethanol; 4 = dichloromethane; 5 = acetone; 6 = isopropanol; 7 = diethyl ether; 8 = tetrahydrofuran; 9 = chloroform; 10 = ethyl acetate; 11 = dioxane; 12 = *n*-butanol; 13 = trichloroethylene; 14 = N,N-dimethylformamide; 15 = hexane; 16 = benzyl alcohol (general solvent).

RESULTS AND DISCUSSION

In the development of a generalized method for the determination of a wide variety of residual solvents in bulk pharmaceuticals, simplification can be achieved through the establishment of conditions for the separation of as many potential solvents as possible. Under such conditions, the method has the following desirable features:

(1) The presence of one solvent in the drug does not interfere with the determination of another.

(2) Multiple solvent determinations are possible from a single injection of a sample solution.

(3) A single set of standards containing multiple solvents can be analyzed for the construction of calibration curves.

(4) The changing of conditions for analyses of different solvents becomes unnecessary.

The use of highly selective chromatographic conditions also reduces the possibility of interferences in a determination by the drug itself or its decomposition products. While such high selectivities can often be achieved through the use of capillary columns, in the present work this is impractical, since high analytical precision with such columns can only be assured by utilizing on-column injection systems[8,9], which are currently very difficult to couple to autosampling equipment. Alternatively, a packed column with the highly selective stationary phase SP1500 on Carbopack B was used. Under the conditions of the determination, this stationary phase was capable of fully resolving 13 of 15 common residual solvents (Fig. 1). The unique selectivity of such graphitized carbon-black stationary phases has been attributed to separation mechanisms based upon geometric differences between the components being separated[3,10,11]. Under the present conditions, boiling point differences also appear to be a factor. When the corrected retention times of the solvents, R, are fit by multiple linear regression to the three-parameter equation,

$$R = \alpha M + \beta T + \nu$$

using both molecular volume, M (*i.e.*, molecular weight divided by density), and boiling point, T, as independent variables and α, β, and ν as constants, good agreement between calculated and experimental values is obtained, as shown graphically in Fig. 2 (correlation coefficient = 0.92).

Optimum precision and accuracy of any gas chromatographic determination is possible only if the sample being analyzed is homogeneous. In the present determination, this is ensured by the preparation of the sample in a solvent which is capable of completely dissolving both the sample and the residual solvent being determined. Additionally, this "general dissolution solvent" should not interfere with the determination of the residual solvent by coelution under the chromatographic conditions. In the present method, benzyl alcohol was chosen as the general solvent since it meets most of these criteria. Virtually all solvents and most pharmaceutical compounds are soluble in it at levels used in this determination. Under the experimental conditions, benzyl alcohol elutes well beyond any of the potential residual solvents, owing to its high boiling point (205°C). While this ensures that the general

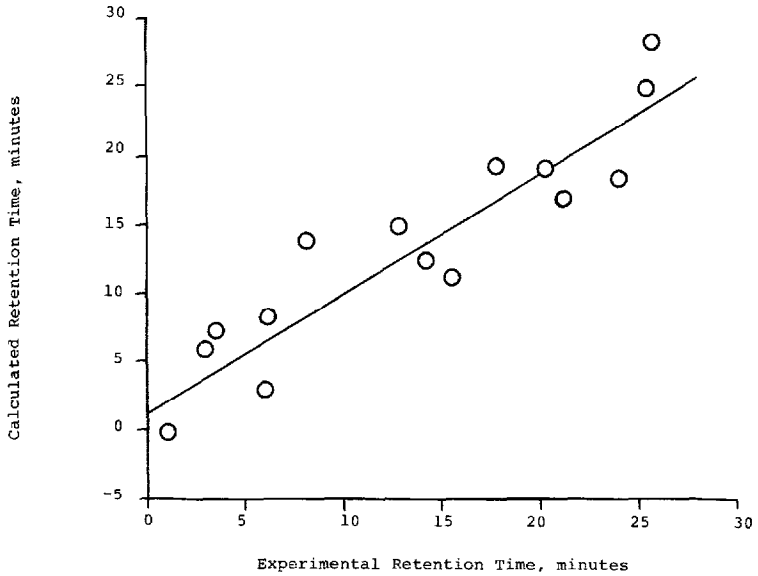

Fig. 2. Plot of predicted corrected retention times of residual solvents by linear model versus experimental values using temperature program number 2. Linear model: $R = 0.31\ M + 0.16T - 23.1$ (see text). Retention times are corrected using butane as an unretained material.

solvent does not interfere with the solvent being determined, it requires that conditions are used which result in the complete elution of the general solvent from the column before any subsequent samples are analyzed. It is for this reason that the final column temperature in the program used for the determination is held for a relatively long period of time (>20 min). Such long "burn-off" periods have been used for benzyl alcohol in other studies, such as the analysis of pharmaceutical compounds for residual solvents by head-space techniques[2].

Although the unreliable reproducibility of manual GC injections often requires the use of internal standards for maximum system precision[12], in the present deter-

TABLE II

SYSTEM PRECISION DATA FROM REPLICATE INJECTIONS OF STANDARDS

Except where noted, standard solutions injected had concentrations of 0.008 mg/ml solvent in benzyl alcohol. R.S.D. = relative standard deviation of peak heights from six replicate injections.

Solvent	*R.S.D.*	*Solvent*	*R.S.D.*
Methanol	1.0	Chloroform*	0.90
Acetonitrile	4.0	Ethyl acetate	1.8
Ethanol	5.2	Dioxane	2.5
Acetone	2.8	*n*-Butanol	0.76
Isopropanol	4.0	Trichloroethylene	0.79
Diethyl ether	1.3	N,N-Dimethylformamide*	1.9
Tetrahydrofuran	1.2	Hexane	2.5

* Standard solution concentration: 0.032 mg/ml.

mination this was overcome by employing an autosampler. Table II displays statistical data obtained from replicate injections of standard solutions containing several potential residual solvents, using such an automatic injection system. Injection precision, as measured by the standard deviations of the chromatographic peak heights, is at acceptable levels for vitually all solvents. The high level of injection reproducibility leads directly to high levels of method precision, as determined by the standard deviations of replicate residual solvent determinations on the representative pharmaceutical compounds (Table III).

Accuracy

The determination of the accuracy of any analytical method is not a simple task. In some cases, such as the assay of a bulk drug against a reference standard, it has been suggested that a simple demonstration of the linearity of chromatographic response as a function of concentration is sufficient to ensure acceptable accuracy[13]. In the present case, however, it appears that more is necessary, since the determination is based upon reference to a calibration curve which is generated from data obtained from standards which do not contain the drug matrix. Effects of the drug or its decomposition products upon chromatographic conditions or reaction of the residual solvent with components of the drug matrix are potential sources of bias which must be considered when evaluating the accuracy of such a determination.

Errors caused by effects of the matrix in chromatographic determinations can be minimized through the use of the method of standard additions, in which known amounts of the substance being determined are successively added to a solution of the drug matrix, and a linear plot of chromatographic response *versus* the concentration added is constructed. The intercept of this plot with the x-axis corresponds to the concentration of the substance in the unspiked solution. Fig. 3 shows an example of such a plot for the determination of N,N-dimethylformamide in a sample of an experimental antitumor drug. Because it requires the construction of a calibra-

TABLE III

RESIDUAL SOLVENT ANALYSIS OF REPRESENTATIVE DRUGS

% = Percentage of residual solvent found in sample; σ = standard deviation of determination; CI = 99% confidence interval for determination; SR = ratio of slope of standard addition curve to external standard curve; H = hypolipidemic; T = antitumor; P = antihypertensive; B = antibiotic; C = anticonvulsive; EtOH = ethanol; DMF = N,N-dimethylformamide; HEX = hexane; THF = tetrahydrofuran; ACN = acetonitrilee; MeOH = methanol.

Drug number	*Drug type*	*Residual solvent*	*External standard (normal) method*			*Standard addition method*			*SR*
			%	*σ*	*CI*	*%*	*σ*	*CI*	
1	H	EtOH	0.23	0.01	±0.02	0.19	0.007	±0.01	0.91
2	T	DMF	0.17	0.01	±0.02	0.23	0.01	±0.02	0.86
3	P	HEX	0.64	0.06	±0.12	0.46	0.13	±0.21	0.98
4	B	EtOH	4.5	0.3	±0.6	3.9	0.9	±1.5	0.76
5	P	THF	0.020	0.001	±0.002	0.020	0.006	±0.01	0.97
6	P	ACN	0	0	0	0	0.015	±0.03	1.06
7	C	MeOH	0.031	0.004	±0.008	0.04	0.02	±0.04	1.03

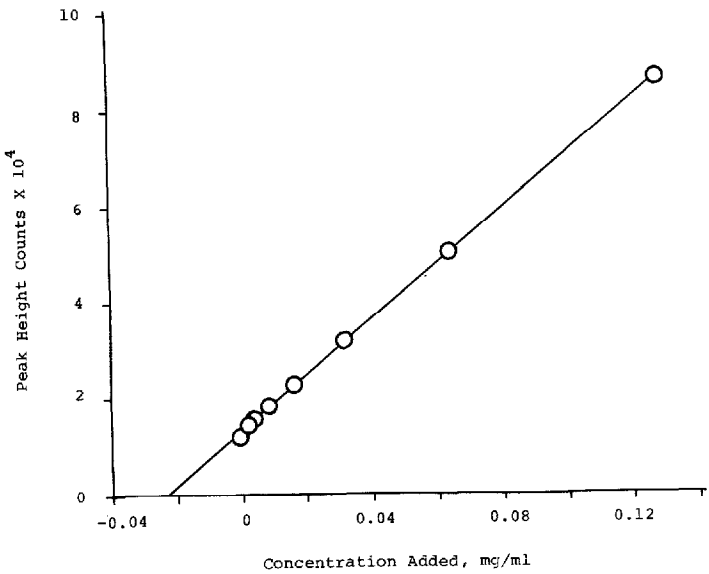

Fig. 3. Standard addition curve for the determination of N,N-dimethylformamide in an antitumor drug (drug No. 2, Table III). Intercept of curve with x-axis corresponds to the concentration of N,N-dimethylformamide in the unspiked solution of the drug in benzyl alcohol.

tion curve for every sample, the standard addition method is not a very efficient technique for the analyses of multiple samples. In the present study, however, comparison of results obtained from standard addition and reference to an external standard curve on representative samples can give an indication of the effect of the drug matrix on the determination.

Massart *et al.*[14] suggest two methods for comparing results obtained from standard addition and external standardization methods:

(1) The slopes of the standard addition and external standard curves are obtained by linear regression and compared. The comparison can be made either by a statistical significance test or, more simply, by calculation of the ratio of the slopes, which has been shown to be directly related to the recovery of the analyte in the standard addition method[15]. A significant difference in this ratio from unity (a 5% difference has been proposed as being significant[16]) is taken to indicate the presence of experimental bias in the external standard curves due to the effects of the sample matrix.

(2) A comparison is made of results of determinations obtained from the standard addition and external standardization methods. This is usually accomplished by a comparison of confidence intervals or a Student t-test.

In the present study, the ratios of slopes of external standard to standard addition curves deviate from unity by more than 5% in four of the seven representative determinations (see Table III), yet the differences in amounts of solvent found by the two methods are minimal, even in the samples with the widest deviation in slope ratio from unity (drug No. 4). Apparently the standard curve slope ratio is a more sensitive probe for detecting matrix effects than is a simple comparison of the results of solvent determinations by standard addition and external standardization.

However, comparison of the results demonstrates that the bias in the determination caused by these matrix effects is not sufficient to make a noticeable difference in the two types of determinations, at least in the compounds under study here. Generally, then, the use of external calibration curves for this type of determination appears to give acceptably accurate results which are comparable to those obtained by standard addition methods.

CONCLUSIONS

Application of a generalized sample preparation procedure and highly selective chromatographic conditions allows the method described here to be used for the determination of a wide variety of potential residual solvents in pharmaceutical compounds of various types. Incorporation of an autosampler eliminates the need for internal standardization and allows for high sample throughput, while the specificity of chromatographic retention times for each solvent gives the method a potential capability for identifying unknown volatile impurities. While detection limits have not been rigorously determined, the method has been shown to give precise and accurate analytical results down to at least 0.02% of each residual solvent by weight.

Although matrix effects were found to be minimal in the representative determinations done here, interferences in a specific determination by a drug or its thermal decomposition products are always possible. When such interferences are suspected, due to the presence of extraneous chromatographic signals or some other anomolous behavior, the statistical methods described here can be used to determine the magnitude of the error caused by such effects. Standard addition techniques can then be applied to the method when such error is considered to be significant.

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