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DETERMINATION OF ALCOHOLS IN PHARMACEUTICALS BY ION-EXCLUSION CHROMATOGRAPHY

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SUMMARY

Alcohols present in pharmaceutical intravenous solutions (*e.g.* propylene glycol and ethanol) are separated from each other and common matrix components by an ion-exclusion column with a dilute sulfuric acid mobile phase. Using refractive index detection, the assay is characterized by an absolute detection limit of 1 ng and a linear dynamic range for both area and height which spans three orders of magnitude. Chromatographic ruggedness is sufficiently high so that strict system suitability criteria can be met even after 600 injections have been made. Long-term detector stability is enhanced through the use of a flowing reference cell.

INTRODUCTION

While short-chain alcohols are not common components of parenteral intravenous solutions, they are occassionally included in formulations to serve as stabilizers and/or solubilizing agents. Their accurate and precise quantitation in these types of samples is important in terms of process control, stability testing and product development. While gas chromatographic (GC) assays exist for the quantitation of both species of interest (propylene glycol and ethanol)[1–5], no generic assay which is readily adaptable for use in pharmaceutical matrices exists for the analysis for these two species in a single injection. Additionally, the GC assays generally require some sample pretreatment (dilution, addition of an internal standard, derivatization). Thus while their sensitivity and selectivity may be high, they may not be as cost-effective as a methodology which is capable of quantitating both analytes in the same injection. One must also be concerned about both the ability of the GC assays to provide interference-free separations in complicated pharmaceutical matrices and the long-term stability of GC columns. Liquid chromatography (LC) represents a viable alternative to GC; indeed, effective columns have been developed for the LC separation of mixtures of carbohydrates and alcohols[6–9]. While the lack of a strong chromophore precludes detection by UV spectrometry, both refractive index and the more sensitive amperometric detection methods are suitable for quantitation of the alcohols. During preliminary evaluation of a commercially available ion-exclusion column, it was observed that an analytically significant separation could be achieved between the an-

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alytes using fairly common elution conditions. The purpose of this paper is to describe the optimization, performance and application of the ion-exclusion method for these two alcohols.

EXPERIMENTAL

Chromatographic system

The chromatographic system consisted of a Waters 501 pump, a Micromeritics Model 728 autosampler interfaced with a Valco VICI injector, a Waters R401 refractive index detector, a conventional strip-chart recorder and a Hewlett Packard 3357 computer integrator. The chromatographic column was a HPICE-AS1 column (25 × 1.2 cm) available from Dionex, An Alltech "Direct Connect" C_{18} cartridge was used as a guard column. The mobile phase of 0.025 *N* sulfuric acid was supplied at a flow-rate of 1.0 ml/min. While sample size and detector sensitivity were varied over the course of the study, for routine applications these quantities were 20 μl and 8 × respectively. The refractometer was operated with mobile phase in the reference cell; use of both a closed and flowing reference cell was examined. In the flowing cell configuration, the mobile phase was supplied by gravity feed. All chromatography was performed at ambient temperature; however, the column and all connecting tubing was wrapped in plastic foam sheeting to provide some thermal insulation. Additionally, the refractometer was mounted on a styrofoam foundation to eliminate vibration noise.

Reagents and samples

Samples used for the characterization of system's performance represent laboratory preparations which contained the two analytes and various species of common pharmaceutical interest. These samples were prepared to contain roughly 1.5–2.5% ethanol and 0.2–0.4% propylene glycol by serial dilution of more concentrated stock materials which were either themselves commercially available or were prepared from reagent-grade salts. Samples containing other potential pharmaceutical matrix components were prepared by this similar manner at the concentration levels listed in Table I. Standards were prepared by dilution of reagent-grade propylene glycol and 200 proof ethanol (USP-grade) in water; the working standard range was 1.5–2.5% for ethanol and 0.6–4.0 mg/ml for propylene glycol. All chemicals used were reagent- or pharmaceutical-grade; nitroglycerin degradates were obtained from Radian (Austin, TX, U.S.A.). Water used in this study was obtained from a Millipore Milli-Q purification system.

RESULTS AND DISCUSSION

The chromatographic behavior of propylene glycol and ethanol is shown in Fig. 1; while total analysis time is somewhat long at 15.5 min, this is primarily related to the large void volume associated with the column used. As shown in Fig. 1, both analytes are readily separated. Additionally, many common pharmaceutical diluents, drugs and their common degradation products show little or no affinity for the resin used under the elution conditions employed (Table I). Only the acetate ion shows any significant retention; however, with a retention time of 9.7 min it is completely

TABLE I

RETENTION CHARACTERISTICS OF COMMON COMPONENTS OF PHARMACEUTICAL SAMPLES

Species	*Level (tested)*	*Retention time (min)*
Void	–	4.2
Citric acid	5 m*M*	5.4
Dextrose	5%	5.6
Nitrated glycerins*	30–400 μg/ml	4.2
Common drugs	2%	4.2–5.4
Sodium chloride	0.9%	4.4
Sodium acetate	5 m*M*	9.7
Sodium phosphate	5 m*M*	5.8
Sodium carbonate	5 m*M*	4.2
Sodium gluconate	5 m*M*	5.7
Propylene glycol		11.3
Ethanol		14.4

* Including tri-, di- and mononitroglycerin.

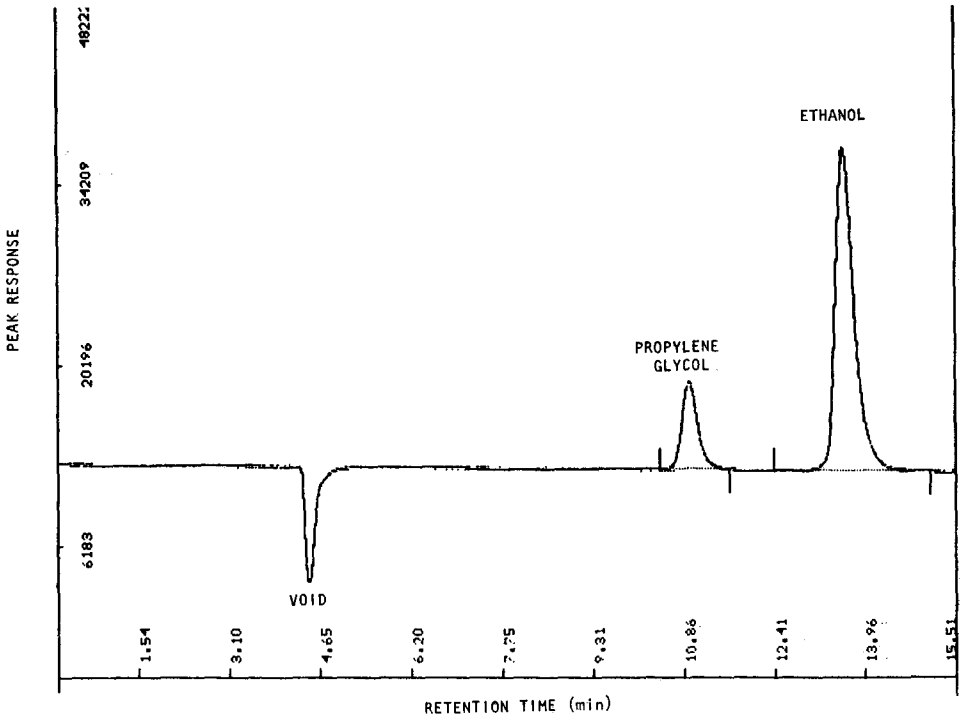

Fig. 1. Chromatographic performance of the alcohol assay. Chromatographic conditions include: column, HPICE-AS1; mobile phase, 0.025 *N* sulfuric acid; flow-rate, 1 ml/min; sample size, 20 μl. Sample shown is the middle standard containing 2% ethanol and 2 mg/ml propylene glycol in a water matrix.

resolved from both the analytes of interest. While the retention mechanism in this method is somewhat complicated, various researchers have suggested that ion exclusion, normal- and reversed-phase partitioning and ion-exchange can all contribute to the behavior exhibited by this chromatographic system[10–13].

The performance of this analytical method can be divided into two categories; ability to quantitate the analytes and ruggedness. Considering the former category, Table II documents recoveries and accuracies obtained when the laboratory prepared formulations containing known quantities of both analytes were characterized with the chromatographic method in three separate experiments (trials). In this Table, A, B and C refer to formulations which contain differing amounts and types of pharmaceutical matrix components while levels of 0.8, 1.0 and 1.2 represent the relative amount of the analytes. A level of 1.0 corresponds to a sample containing 35 mg/ml propylene glycol and 2% ethanol. Recovery represents the percent ratio of measured concentration *versus* actual preparation concentration and falls in the range of 98 to 101% for both species. No statistical bias is seen in the overall recovery data and neither matrix nor analyte level affect accuracy. Precision, defined as the reproducibility of multiple injections of a sample made within a given analytical run, is typically less than 1.5% relative standard deviation (R.S.D.) for both analytes. Since there is no sample preparation involved in the method (other than dilution should the analyte levels be higher than the standard range) intra-run precision is on the same order of magnitude as inter-run precision. The refractive index detector is suitabily sensitive

TABLE II

SUMMARY OF RESULTS OF THE ALCOHOL ASSAY

Matrix	*Level*	*Propylene glycol*		*Ethanol*	
		Recovery (%)	*R.S.D. (%)*	*Recovery (%)*	*R.S.D. (%)*
Trial 1: preliminary study (A)					
A	0.8	100.1	1.5	101.2	0.3
A	1.0	98.6	1.3	100.5	0.1
B	1.0	100.6	0.4	99.6	0.1
C	1.2	99.9	0.6	100.1	0.1
Trial 2: preliminary study (B)					
A	0.8	98.7	0.9	100.0	0.2
A	1.0	98.8	0.6	99.5	0.6
B	1.0	100.5	1.5	100.0	0.6
C	1.2	100.1	0.8	98.5	0.4
Trial 3: extended study					
A	0.8	97.8	0.6	99.0	0.4
A	1.0	99.8	0.9	99.5	0.2
A	1.2	99.6	0.4	101.5	0.6
B	0.8	99.7	0.8	101.0	0.2
B	1.0	100.6	0.8	100.5	0.2
B	1.2	100.3	0.4	100.5	0.2
C	0.8	100.1	1.8	99.5	1.4
C	1.0	100.4	0.4	99.5	0.3
C	1.2	100.3	0.4	99.0	0.3

for the desired application; detection limits for propylene glycol and ethanol are 5 μg/ml and 0.003% respectively. For ethanol, both peak height and area response is linearly related to analyte concentration over the concentration range of 0.005 to 4%. For propylene glycol, both area and height show a linear relationship to concentration in the range of 0.005 to 10 mg/ml.

Ruggedness, as used in this manuscript, includes two components; chromatographic performance and response stability. Considering the former component, Figs. 2–5 document the performance of one chromatographic column as a function of the number of injections made onto it. Parameters of interest include resolution (between both analytes), theoretical plate count, capacity factor and tailing factor. Resolution, capacity factor and tailing factor were all constant over the course of nearly 700 injections; only theoretical plate count revealed a declining column efficiency. Similar behavior was exhibited by two additional AS-1 columns. This type of performance results in the adoption of the system suitability criteria documented in Table III; it is common practice that any chromatographic system to be employed for sample analysis must meet (or exceed) these criteria before it can be used for sample analysis. It is these authors' experience that typical AS-1 columns can easily meet these stringent requirements for over 800 injections.

In addressing detector stability, we require that the response be stable throughout an analytical run as well as from run-to-run. Intra-run stability is particularly important in our applications wherein productivity is maximized by making analytical runs which last for more than 12 h. While it is our experience that the refractive index detector exhibited an equivalent response on a day-to-day basis, we found that

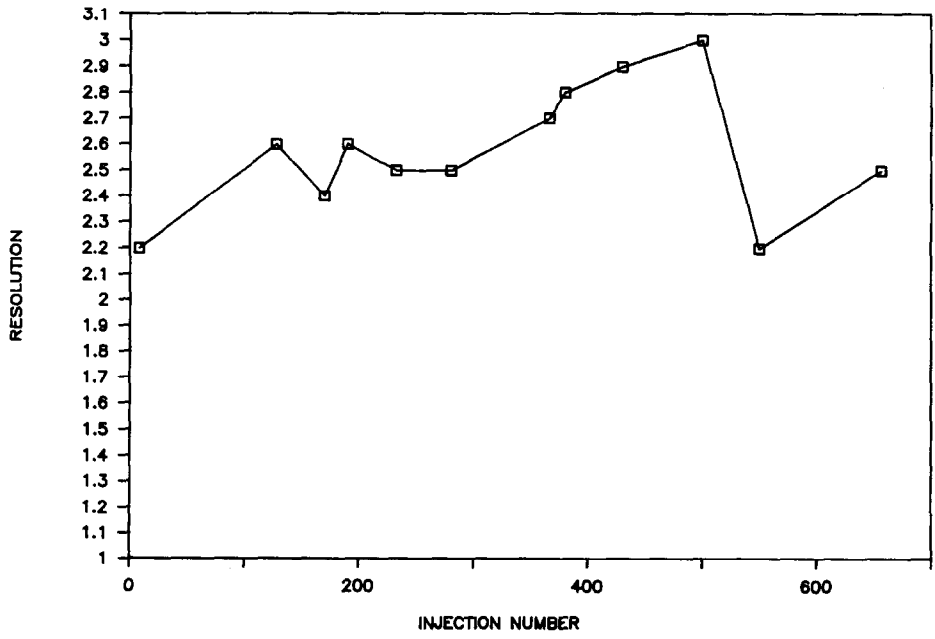

Fig. 2. System's performance, chromatographic ruggedness. Resolution (between the two analytes) as a function of injection number onto a single column.

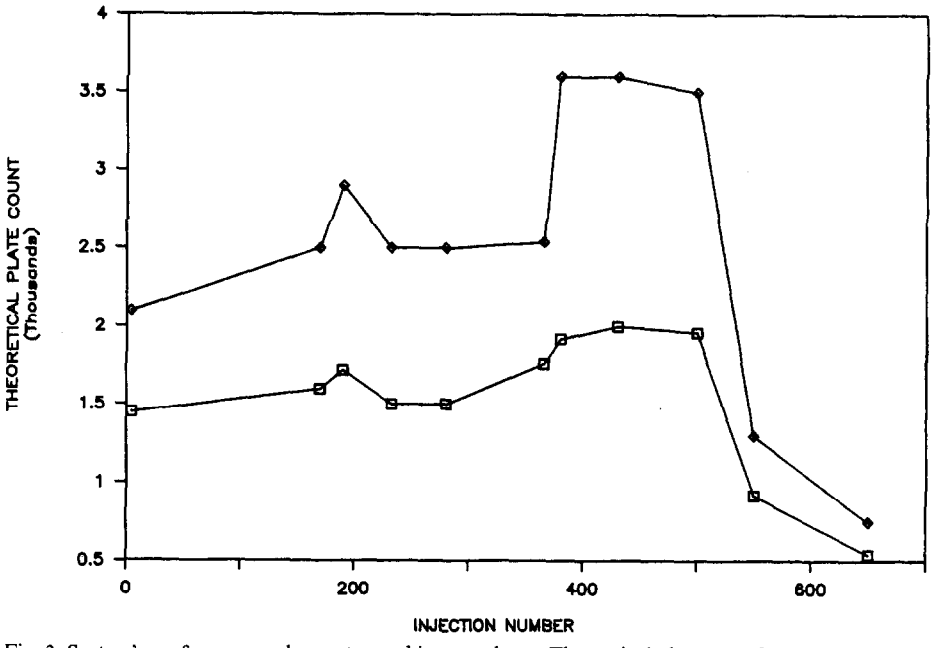

Fig. 3. System's performance, chromatographic ruggedness. Theoretical plates as a function of a injection number onto a single column. □ = Ethanol; ◇ = propylene glycol.

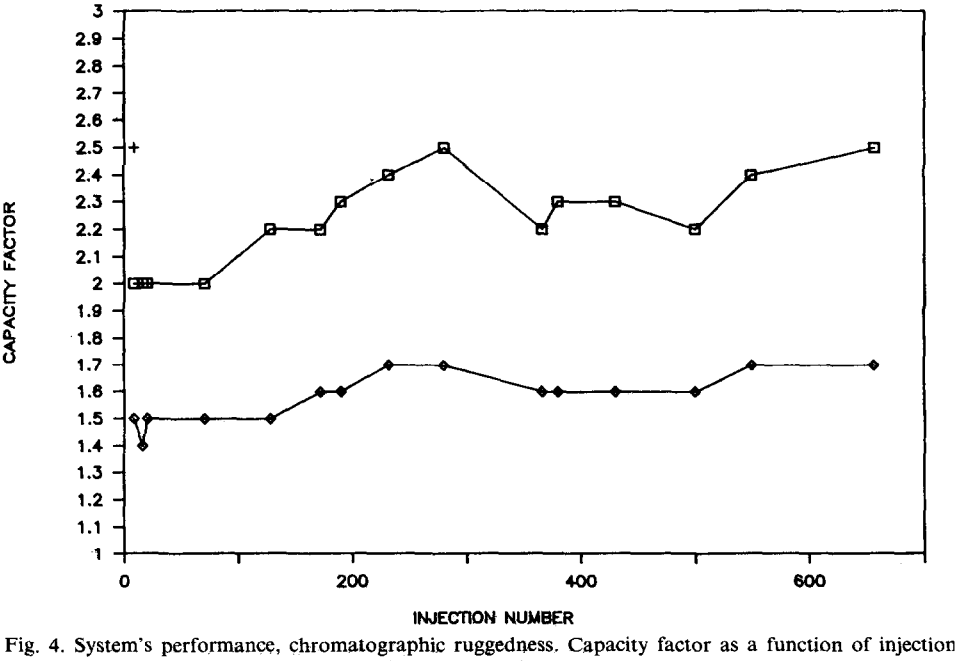

Fig. 4. System's performance, chromatographic ruggedness. Capacity factor as a function of injection number onto a single column. See Fig. 3 for key to symbols.

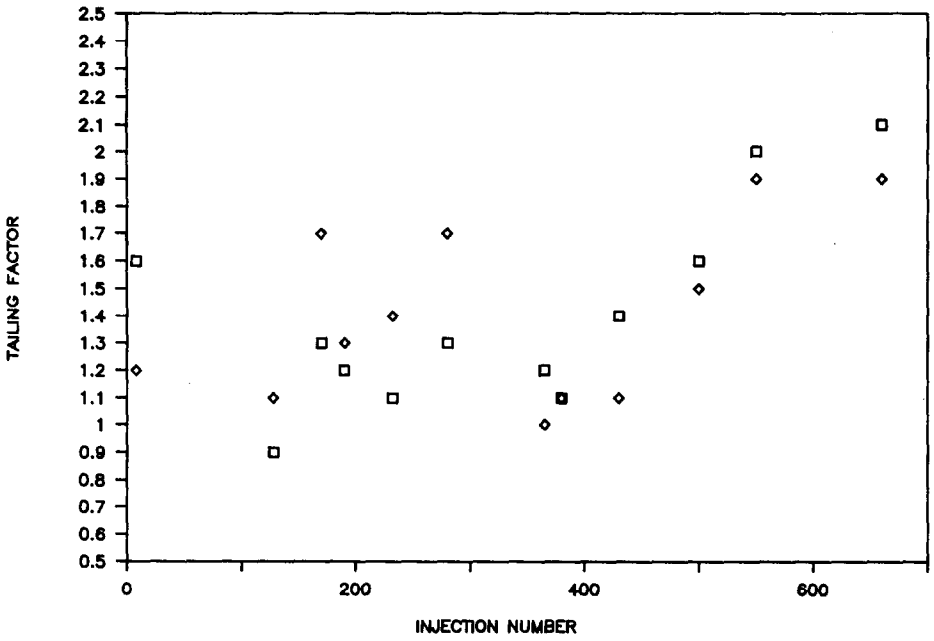

Fig. 5. System's performance, chromatographic ruggedness. Tailing factor as a function of injection number onto a single column. See Fig. 3 for key to symbols.

when a static reference cell (filled with the mobile phase) was used, a considerable intra-run drift in both the baseline signal and response factor was routinely observed. The isolation of the detector and the nature of the drift was such that vibration and thermal variations were eliminated as potential causes of these effects. It is felt that the source of the drift is a change in the concentration of the solution in the reference cell caused by solvent evaporation. Thus, the use of a flowing reference cell, in which mobile phase is supplied to the cell at a low flow-rate by gravity feed, was projected

TABLE III

SYSTEM SUITABILITY CRITERIA

Property	*Criteria*	
	Propylene glycol	*Ethanol*
Capacity factor	> 1.3	> 1.8
Tailing factor	< 2	< 3
Theoretical plates	> 1400	> 1000
Resolution*	> 2	
Precision**	< 2	< 1.5
Linearity***	> 0.999	

* Between the two analytes.
** R.S.D. (%) of triplicate injections.
*** Correlation coefficient of calibration curve.

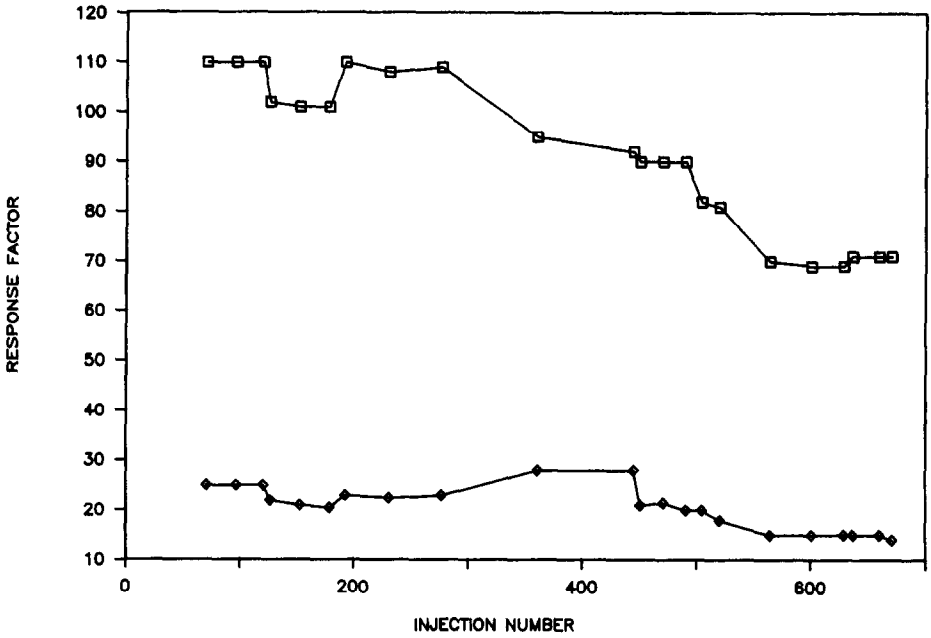

Fig. 6. System's performance, detector stability. Area response factor as a function of injection number onto a single column. The response factor is defined as the product of the peak area and the detector attenuation divided by the sample size. The middle standard is used for the calculation of all performance criteria. See Fig. 3 for key to symbols.

as a suitable solution. Upon implementation of this approach, the deviation in both area and height response observed over the course of runs lasting in excess of 18 h was lessened so much as to be statistically insignificant. As shown in Fig. 6, detector response can be stabilized over large periods of time spanning multiple analytical runs through the use of such a flowing reference cell.

CONCLUSION

The chromatographic method described herein, which uses ion-exclusion chromatography coupled with refractive index detection, is suitabily accurate, precise and rugged to be used to support research and development efforts, process control and quality control insurance functions with respect to the determination of the ethanol and propylene glycol content of typical pharmaceutical formulations.

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