A Technique for Manual Injection to Capillary Columns with Avoidance of Sample Discrimination

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ABSTRACT: When sample is applied to a capillary column by any one of a number of automated injection systems, it is possible to achieve high standards of analytical accuracy. In general, it is much more difficult to achieve high-quality results with manual injection. Three injection-related problem areas are discussed that can adversely affect analytical accuracy, more so with manual than with automated injection, *viz.*, fractionation from the syringe needle, overloading of the injector insert with vaporized sample, and loss of sample past the seal between the syringe barrel and the needle. Measures have been detailed to reduce the loss of accuracy caused by these phenomena. The injection technique described is slow enough that it and human capabilities are compatible, the sample is re-focussed on the column, so that there is little or no resultant loss of column efficiency and resolution, and, therefore, high accuracy results are obtainable.

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From the inception of capillary gas chromatography, and during its subsequent developmental period, the most commonly used method of sample introduction has been the split injection technique. Perhaps the most important advantage of this technique is that, because the time during which sample is applied to the column is so short, it causes only an insignificant contribution to band broadening of the separated peaks. Thus, with respect to both resolution and peak shape, chromatograms obtained following a split injection are intrinsically of the highest possible quality that the column can produce. It is a simple operation to carry out a split injection and to control the split ratio, and this is commonly perceived to be an advantage. However, this simplicity of execution is not as advantageous as it might seem at first, because it is an extremely difficult task to optimize the whole of a split injection system so as to avoid sample discrimination, and thereby to achieve a linear sample split. As a consequence, when a split injection is employed, the results obtained can be highly and unpredictably inaccurate (1). This problem has long been recognized, and many papers have been published on the problems. In their 1987 paper on split injection to capillary columns, Bannon *et al.* (2) reviewed a number of the more important publications that had appeared to that date on sample discrimination. They also detailed a method for the optimization of a split injection system so that highly accurate and repeatable results could be obtained for the analysis of fatty acid methyl esters (FAME) when using a high-speed automatic liquid sampler. Three factors were identified by these authors (2) as critical for high accuracy analysis, *viz.*: (i) very rapid sample injection as an effective means of avoiding syringe needle discrimination; (ii) rapid vaporization of the sample, achieved by the combined use of dilute solutions of analyte in the solvent, the smallest sample size commensurate with the acquisition of a chromatogram that could be accurately quantitated, the use of a vaporization temperature higher than that generally adopted, and a high thermal-capacity packing in the injector insert; and (iii) intense mixing of the vaporized sample with the carrier gas prior to splitting, achieved by careful attention to the design of the injector insert.

Other factors were identified, but their importance was found to diminish as those three factors were progressively optimized. In this work, speed of injection and consequent avoidance of sample discrimination were achieved by the use of a Hewlett-Packard (Palo Alto, CA) Model 7673A high-speed automatic liquid sampler, whereby injection was accomplished in approximately 0.1 s.

As an alternative to high-speed injection, Schomburg *et al.* (3) advocated an injector in which the syringe needle was kept cold, while the sample was injected to a heated zone. Their published results were of excellent accuracy. A third approach is to use a programmed temperature vaporizer, whereby the sample is injected into a cold system, and then rapidly heated to effect vaporization. The Optic Temperature Programmable Injector, Model 126000 (Ai Cambridge Limited, Cambridge, United Kingdom), is a typical commercially-available injector that employs this approach.

Each of these three techniques to avoid sample discrimination requires expensive equipment and, for small laboratories with limited budgets, none may be a viable option. Such laboratories must rely on the option of manual injection, and, to this author's knowledge, no manual injection technique has

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been devised that allows one to reliably achieve the same accuracy as that obtained by any of the more automated injection methods. In the present paper, it is shown that sample discrimination can occur from the syringe and injection zone by three distinct mechanisms, and measures are described to minimize each of these differential losses. A manual injection technique is detailed that is slow enough to be compatible with human capabilities, allows preservation of chromatographic resolution and peak shape, and gives results of high accuracy for the analysis of FAME.

EXPERIMENTAL PROCEDURES

Primary standard mixture. A primary standard mixture of the even-numbered saturated FAME from 8:0 to 18:0 was made from high-purity (*ca.* 99%) reference esters (Sigma Chemical Co., St. Louis, MO) by the procedure of Albertyn *et al.* (4). Solutions of this mixture were made up in reagent-grade isooctane (2,2,4-trimethylpentane) at concentrations of 3, 2, 1, and 0.5% (wt/vol). The percentage composition of the standard was: 8:0, 8.55; 10:0, 6.62; 12:0, 46.73; 14:0, 18.94; 16:0, 9.13; and 18:0, 10.03.

Syringe testing technique. A 10-μL SGE syringe Cat. No. 002000 10F (SGE Pty Ltd., Ringwood, Australia) was used. Five μL isooctane was introduced into the syringe, which was then inserted into the heated injector at operating temperature and pressure, but the plunger was not depressed. High pressure of the inlet causes the solvent to be pushed back, and the time taken to empty the syringe backwards should exceed 1.5 min at a head pressure of 123 kPa.

Syringe filling and injection technique. The syringe barrel and needle should be filled with isooctane, removing any air bubbles and depressing to the 2-μL level. Successively draw into the syringe, 0.5 μL air, 1.0 μL sample solution, 0.5 μL air, 2.0 μL isooctane, finally sufficient air that the whole of the liquid contents of the syringe are visible in the barrel of the syringe (Fig. 1). After inserting the syringe into the heated injection zone, the plunger should be depressed slowly and as evenly as possible during about 5 s. The syringe needle should be left in the injection zone for a further 5 s.

Injector parameters. We used an injector insert (Hewlett Packard Part No. 5062-3587) packed successively with 2 mm silica wool, 30 mm 3% SE30 on 80-100# Gas Chrom Q (Supelco Inc., Bellefonte, PA), 3 mm silica wool, the composite packing being compressed firmly enough that the syringe needle can penetrate easily but without dislodging the packing. The insert was positioned in the inlet housing upside down, i.e., with the restricted end uppermost and the open end over the sample split point (Fig. 2); injector temperature 350°C; septum purge flow rate *ca.* 2–3 mL/min.

FIG. 1. Syringe-filling technique. Black sections in barrel are solvent or sample solution; clear sections represent air.

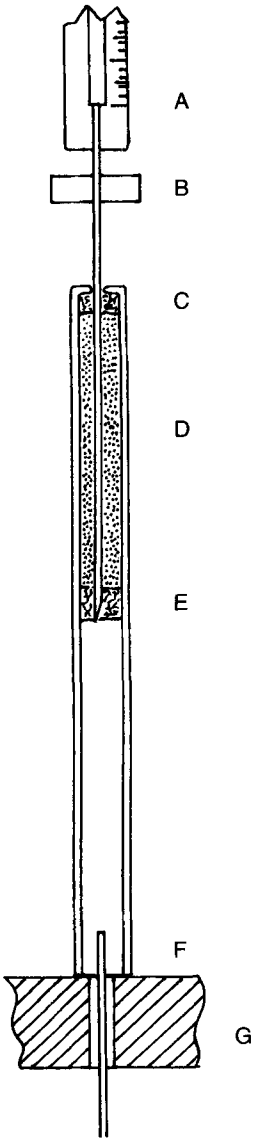

FIG. 2. Injector insert. A, syringe; B, septum (septum support not shown); C, 2-mm silica wool; D, 30 mm 3% SE30 on 80-100# Gas Chrom Q; E, 3 mm silica wool at injection point; F, capillary column 5 mm into insert; and G, firewall between injector and column ovens.

Other chromatographic parameters. Gas–liquid chromatography was carried out on a Hewlett-Packard Model 5890 gas chromatograph fitted with a flame-ionization detector; 60 m × 0.25 mm i.d. fused-silica column coated with 0.25 μm DB23 (50% cyanopropyl/50% methylpolysiloxane; J&W Scientific, Folsom, CA); the end of the column was inserted 5 mm into the heated injector (Fig. 2); hydrogen carrier gas; column inlet pressure 123 kPa; column flow velocity 32 cm/s (=0.93 mL/min); total hydrogen flow rate to the detector 30 mL/min; make-up gas, nitrogen, flow rate 24 mL/min; air flow rate, 300 mL/min; detector temperature, 250°C; column oven temperature program, 130°C for 0.5 min, 40°C/min to 180°C, then held isothermally at this temperature. Raw peak areas were measured by a Hewlett-Packard Model 3392 computing integrator and were corrected by applying the theoretical relative response factors of Ackman and Sipos (5), as tabulated by Craske and Bannon (6), which were: 8:0, 1.1927; 10:0, 1.1233; 12:0, 1.0771; 14:0, 1.0440; 16:0, 1.0193; and 18:0, 1.0000. Split flow was varied through the range of 120 to 20 mL/min, which corresponds to split ratios from 129 to 22, as detailed later.

Evaluation of analytical results. The absolute error of each FAME was determined, and, from these figures, the "grade of analysis" was obtained as previously defined (2,7).

RESULTS AND DISCUSSION

As one of the team that developed the method of optimizing split injection to a capillary column when using a high-speed automatic injector (2), it came as a surprise to find that, when required to change to an instrument that was equipped only for manual injection, it was not possible to obtain the high quality of result that had been previously obtained as a matter of routine when using generally accepted techniques of manual injection. Bannon *et al.* (2), using a primary standard of known composition, demonstrated that it was possible to achieve a "grade of analysis," as defined by Herb *et al.* (7), of 99.5 or better. By contrast, the present author, using manual injection but otherwise following the recommendations of Bannon *et al.* (2) for instrument optimization, has been able to achieve grades of analysis only within the general range 94–99 (results not reported). As the only significant difference was the method of injection, it seemed reasonable to suspect that the lower quality of results obtained was the result of sample fractionation on injection, or loss of sample from the syringe or injection system.

There are three mechanisms that can be envisaged whereby sample can be lost from the injector, or by which a nonrepresentative sample can be injected onto the column: (i) During the time that the syringe remains in the hot injection zone, analyte solution that is left in the needle after the plunger is depressed can be boiled into the injector insert, and, if a fractional distillation occurs, the composition of the material so applied to the column will not be representative of the original sample. (ii) If a large volume sample is applied rapidly to the injection system, the volume of gas formed will exceed the volume of the injector insert and, unless the velocity of the split stream is very high, an amount of the sample must flow back from the top of the insert and be lost down the septum purge line. (iii) If the sample injection rate is slow, and if the seal between the plunger and the syringe barrel is worn to any extent, an amount of sample can be forced back up the syringe by the high pressure of the chromatographic system and so be lost from the analysis. Clearly, each of these phenomena will lead to a loss of analytical accuracy. It is equally clear that the potential for errors of this nature to occur is higher when injecting manually than when any of the automated systems are used. The injection technique detailed in the Experimental Procedures section is designed to minimize the effects of these errors, to cater for the slow speed of manual injection, and, because slow injection can allow band broadening of early eluting peaks, to re-focus the sample on the column so as to produce sharp chromatographic peaks.

Syringe-filling technique. The aim of the syringe-filling technique is to sandwich the analyte solution between two slugs of solvent and to inhibit intermixing by separating each by a short column of air. When filling the syringe, the lower slug of solvent washes the sample into the barrel of the syringe so that, on injection, no trace of analyte can enter the hot injection zone until the plunger is depressed. On injection, the upper slug of sample chases the sample into the injector zone and, by leaving the needle in the hot zone for five seconds, all of the sample is boiled from the needle. Although this technique minimizes needle fractionation, it creates two other problems that must then be addressed, *viz.*, the potential for sample to overload the injector insert, and for band broadening of the early eluting peaks.

Overloading of the injector insert. Because the recommended technique demands injection of a large volume of sample and solvent, it is easy to overload the injector insert with gaseous sample. It was found necessary to inject much slower than normal, and to employ a relatively high split ratio, so that the flow of analyte is toward the split region rather than back to the top of the injection zone. At optimum split ratio (discussed later), an injection time of about 5 s was found to be optimal. At faster rates of injection, there is the risk of overloading the injection insert. At slower injection rates, it is difficult to inject evenly, and often the early peaks split into two. Two additional measures were found beneficial to impede the back flow of sample. First, the insert was reversed so that the restricted end was uppermost; accordingly, the needle partly blocked the insert and impeded the upward flow of gaseous analyte. Second, the upper part of the insert was packed with a particulate stationary phase, thereby providing a chromatographic barrier above the point of introduction of the sample. A similar technique was employed by Bannon *et al.* (8), when analyzing butterfat FAME, to inhibit the back diffusion of the very low molecular weight esters that are characteristic of this fat.

Sample refocusing. Whereas the measures detailed here resolved the back flow problem, the slow injection technique created the problem that the early eluting peaks were broad

because of the long time of sample application. This problem was resolved by reducing the temperature of the column oven while the sample was applied, thereby refocusing the sample on the column. After application of the sample, the chromatograph was rapidly programmed to operational temperature for execution of the analysis. A reduction of temperature of 50°C below operational temperature was adequate to effect good refocusing of early peaks. When the initial temperature was reduced further than this, the baseline at the beginning of the chromatogram wandered excessively during the initial temperature program. Refocusing was inadequate with a lesser temperature reduction.

Syringe quality. No matter how good the seal is between the syringe barrel and the needle, the high pressure of the chromatograph will, in time, force the sample out of the back of the syringe. It is thus necessary that the seal be of sufficiently high-quality that only a negligible amount of the syringe contents will be lost while the injection is being carried out. This phenomenon becomes important when slow injection is called for. For the syringe used in this work, it took 1 min and 45 s to expel 5 μL solvent when the syringe was left in the injection port at a pressure of 123 kPa. With a second, older syringe, the solvent survived only 17 s. The results obtained when this syringe was used were highly variable and of unacceptable quality (results not reported).

For the filling technique adopted for this work, the small amount lost from a good quality syringe is solvent, hence analytical accuracy is not compromised.

Injection technique. One other concept deserves attention. An inherent advantage of automated injection is that the syringe always passes vertically through a single point in the septum, thereby minimizing the tendency to cut out small particles of rubber that can contaminate the top of the injection zone. For the technique now described, as with all manual injection techniques, it is important to try to penetrate a single point of the septum and to keep the syringe vertical to minimize the possibility of the rubber tearing. Prior to application of samples, the insert was prepared for use by slowly inserting an empty syringe needle through the packing, and then inspecting to determine that the packing had not been dislodged. Once prepared in this manner, optimally tamped packings have been found to retain their integrity, but occasional inspection is recommended.

To evaluate the concepts described in this paper, the standard mixture was made to concentrations ranging from 0.5 to 3.0%, and these were injected at split ratios that varied from 22 to 129, so that, for each condition, the total amount of FAME transferred to the column for analysis was approximately constant (Table 1).

The results of Table 1 fell within the limits of 99.16 and 99.70 (average = 99.36; SD = 0.17), and the relationship between grade and split ratio was:

$$\text{grade} = 0.00156 \times \text{split} + 99.260 \; (r = 0.372) \qquad [1]$$

Because of the low correlation coefficient, it would seem unreasonable to draw firm conclusions from this relationship. Clearly, there remains considerable variability between repeat analyses due to problems of manual injection. However, there is slight evidence that grade is improved with increase of split ratio, which is consistent with the concept that higher gas flow through the injection system should be a favorable factor in preventing loss of sample by back diffusion.

This finding differs from that of Bannon *et al.* (2) who found that, with increasing split flow, accuracy passed through a maximum and then declined again.

There are a number of reasons why the findings of the present work might differ from those of Bannon *et al.* (2): (i) Both the design of insert and the method of applying sample differed significantly from those reported previously (2). (ii)

TABLE 1
Analysis of Standard Mixture at Varying Split Ratios and Analyte Concentrations

Analysis number	1	2	3	4	5	6	7	8	9	10	11	12
Total FAME%[a]		3.0			2.0			1.0			0.5	
Split ratio		129			86			43			22	
Percentage composition												
8:0	8.62	8.54	8.59	8.55	8.53	8.63	8.52	8.75	8.46	8.62	8.55	8.34
10:0	6.71	6.68	6.70	6.67	6.70	6.72	6.68	6.78	6.67	6.72	6.68	6.65
12:0	46.45	46.47	46.51	46.39	46.60	46.48	46.35	46.61	46.43	46.6	46.41	46.52
14:0	18.88	18.92	18.92	18.96	18.94	18.88	18.92	18.85	18.94	18.88	18.89	18.98
16:0	9.20	9.22	9.19	9.25	9.16	9.19	9.26	9.04	9.27	9.16	9.23	9.28
18:0	10.14	10.18	10.09	10.18	10.07	10.10	10.26	9.98	10.23	10.02	10.23	10.22
Absolute error												
8:0	0.07	−0.01	0.04	0.00	−0.02	0.08	−0.03	0.2	−0.09	0.07	0.00	−0.21
10:0	0.09	0.06	0.08	0.05	0.08	0.10	0.06	0.16	0.05	0.10	0.06	0.03
12:0	−0.28	−0.26	−0.22	−0.34	−0.13	−0.25	−0.38	−0.12	−0.3	−0.13	−0.32	−0.21
14:0	−0.06	−0.02	−0.02	0.02	0.0	−0.06	−0.02	−0.09	0.00	−0.06	−0.05	0.04
16:0	0.07	0.09	0.06	0.12	0.03	0.06	0.13	−0.09	0.14	0.03	0.10	0.15
18:0	0.11	0.15	0.06	0.15	0.04	0.07	0.23	−0.05	0.20	−0.01	0.20	0.19
Grade	99.33	99.41	99.51	99.32	99.70	99.38	99.16	99.28	99.23	99.59	99.26	99.16

[a]FAME, fatty acid methyl esters.

Had a wider range of split ratios been investigated, a similar point of maximum accuracy might have been discerned. Such an extension of the range was not contemplated, as it was felt that the split ratios selected covered the range most commonly used in practice. (iii) Because of the lower repeatability of manual injection, it remains more difficult to determine trends than is the case with automated injection.

Grades higher than the average were obtained for two of the results reported (injection numbers 5 and 10). The failure to obtain very high grades for the majority of the injections reported indicates that, for those cases, there was still some loss of sample from the injection zone, and that there is still the potential to improve.

Confirmation of this thesis was obtained when the injection time was slowed down even further (10 s injection + 10 s in port). For a number of these very slow injections, the grades fell within the range 99.60–99.76, and when grades of this caliber were achieved, all FAME eluted as single peaks. However, there were other times when, because of the difficulty of injecting steadily during 10 s, the early peaks split into two or three peaks. On these occasions, the grade of analysis was much lower. Clearly, there is a practical human problem associated with slow manual injection.

From this work it is evident that unless care is taken to optimize injection technique, there is considerable potential to lose sample from the injection zone, thereby adversely affecting the accuracy of analyses. Much can be done to overcome the problems identified, even when it is necessary to employ manual injection, but there is still the potential to reach further improvement. Although the measures detailed were designed specifically for manual injection, it is likely that the principles enunciated might also be applicable to an automated injection technique.

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