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New on-column GC sample injection techniques: Making holes in separation capillaries

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Abstract

Cold on-column capillary injection is a precise and convenient way of sample insertion in capillary gas chromatography analysis especially when samples contain thermally labile analites or mixtures with components showing very different volatilization properties, yielding injector analite discrimination for the high boiling substances. On-column injection avoids these problems but the method requires dedicated injector hardware is not accessible to all GC equipment. It would be advantageous and convenient having adequate methods for the reversible conversion of the common split/splitless injectors in the on-column mode. We have successfully implemented two solutions to such a problem, one of them by building a liner with a funnel and the other involving the making of a hole in a 0.53mm quartz capillary guard column. Both methods allow the easy cold on-column injection with a modified capillary needle syringe on common split/splitless injectors.

Keywords: on-column GC injection; capillary hole.

Resumo

A injeção em cromatografia gasosa no interior da coluna capilar a frio (on-column) é uma maneira precisa e conveniente de introdução da amostra, especialmente em análises com amostras que contêm analitos termicamente instáveis ou misturas com componentes que mostram propriedades de volatilização muito diferentes, dando origem a discriminação na evaporação para as substâncias de elevado ponto de ebulição. A injeção "on-column" evita estes problemas, mas o método requer equipamento específico de injetor que não está disponível para todos os equipamentos GC. Seria vantajoso e conveniente ter métodos adequados para a conversão reversível do injetor comum do tipo divisor/não divisor no modo "on-column". Implementaram-se com sucesso duas soluções para este problema, uma delas através da construção de um funil e a outra envolvendo a execução de um furo em pré-coluna de quartzo de 0,53 milímetros. Ambos os métodos permitem a injeção "on-column" com uma seringa normal modificada com agulha capilar no comum injetor divisor/não divisor.

Palavras-chave: injeção em coluna; furo em capilar.

1. Introduction

Gas chromatography (GC) methods such as biodiesel composition or waxes in olive oil analysis are methods that require the use of the cold on-column injector. In this and other techniques that use as separation medium capillary columns such as capillary electrophoresis (CE) the sample injection is a difficult operation taking in account the need of precise sample introduction inside the small bore tube capillaries.

In cases where the sample components can be thermally vaporised in equal conditions and without degradation, the use of the common split/splitless injector is adequate. But if mixture contains components with a large range of boiling points, difficulty in using this injector arises because of the different vaporization rate of the components leading to analite discrimination, resulting in an analysis enriched in the low boiling components[1]. Techniques envised to diminish this problem were developed but are of difficult practical implementation with normal gas chromatographs[2].

These effects can be overcome by depositing the sample mixture inside the capillary wall at low temperatures, which constitutes the so called cold on-column injection method[2-4]. The technique is challenging since the sample has to be placed inside the very small bore capillary tube. Commercially there exist such injector to adapt to specific GC, but often such type of injector is not available for the equipment existent in laboratories.

We aim the implementation of the biodiesel and wax in olive oil analysis methods which requires the cold on-column injector, but the suitable hardware was not commercially available for our laboratory GC's. We have made modifications based on the common split/splitless GC injector that allow the cold on-column injection. Both approaches involve the correct alignment of a modified capillary needle syringe into the interior of a large bore 0.53 mm capillary guard column, to make the desired sample deposition inside the tube wall. The method involves minor modifications on common split/splitless injectors and does not involve any change on gas connections, turning easily the reversible change of injector type. The first approach was developed by designing a special feature funnel liner which can make the correct alignment of the syringe needle to the interior of the capillary. Some difficulties with this technique led us a consider a second approach which consists of placing the top end of the 0.53 mm capped capillary guard column in a position above the GC rubber septum to ensure easy needle insertion and making a hole in the same guard column in a position inside the injector to allow the normal carrier gas flow. These methodologies were successfully assayed with analysis of synthetic and authentic biodiesel samples under the conditions described in the EN 14105 method[5-6].

2. Experimental

2.1. Reagents

Solvents, n-hexadecane and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich and were used as received.

2.2. Equipment

GC analysis were carried out on a Varian 3900 chromatograph fitted with a split/splitless injector trough methods 1 and 2 (see Results). In both injection methods the column set was built up by connecting an uncoated deactivated column gap (2.5 m x 0.53 mm i.d., Phenomenex) to the analytical column (Phenomenex Zebron ZB-5HT; 15 m x 0.32 mm id, df = 0.25 μm), carrier helium at 80cm/s; "split" flow at 10 mL/min, oven program: initial temperature of 50ºC and then ramp at 12ºC min to 380ºC and hold for 10 min; detection with flame ionization detector at 390ºC. Injector was maintained at low temperature (50ºC). The columns were connected trough a GC universal quartz capillary column union (Phenomenex AGO-4716). On-column 1μL samples were injected trough a 10 μL replaceable needle syringe (SGS) to which a 20 cm x 0.20 mm i.d. (o.d. of approximately 0.32 mm) fused silica capillary was adapted as a needle and fixed through a piece of silicone rubber and a spring (See Figure 1-c).

2.3. Sample preparation

100 mg of waste oil biodiesel was derivatized with 100 μL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) for 10 min at ambient temperature in a closed 10 mL vial. After the period of time, 5 mL of n-heptane was added and the solution is ready for injection.

3. Results and Discussion

The normal split/splitless modifications here described involves the use of an empty deactivated guard column (1-2 m of 0.53 mm quartz capillary tube) as a ground to do the on-column injections. The relatively large bore guard column allows that a modified syringe needle can enter inside the 0.53 mm tube which allows the sample deposition in the guard column tube.

For the process to be successful it has to be a very good alignment in the insertion of the small bore capillary needle syringe inside the larger capillary, under normal gas flow conditions existent inside injector. The first technique assayed was by making a liner with a small funnel inside, a system capable of capillary syringe needle orientation to interior of the guard column (see Figure 1-a). The funnel is cut in such a way that the guard column capillary quartz tube end (polyimide free) has a precise contact inside the funnel bottom (see Figure 1-a). Three holes in the body of the funnel allow the normal "split" flow (position 6, Figure 1-a) which is optional but in our case was maintained for removing eventual solvent/ analites in injector as can occur on sample vaporisation reflux or air which can enter in the process the needle insertion/retraction. A similar approach can be made

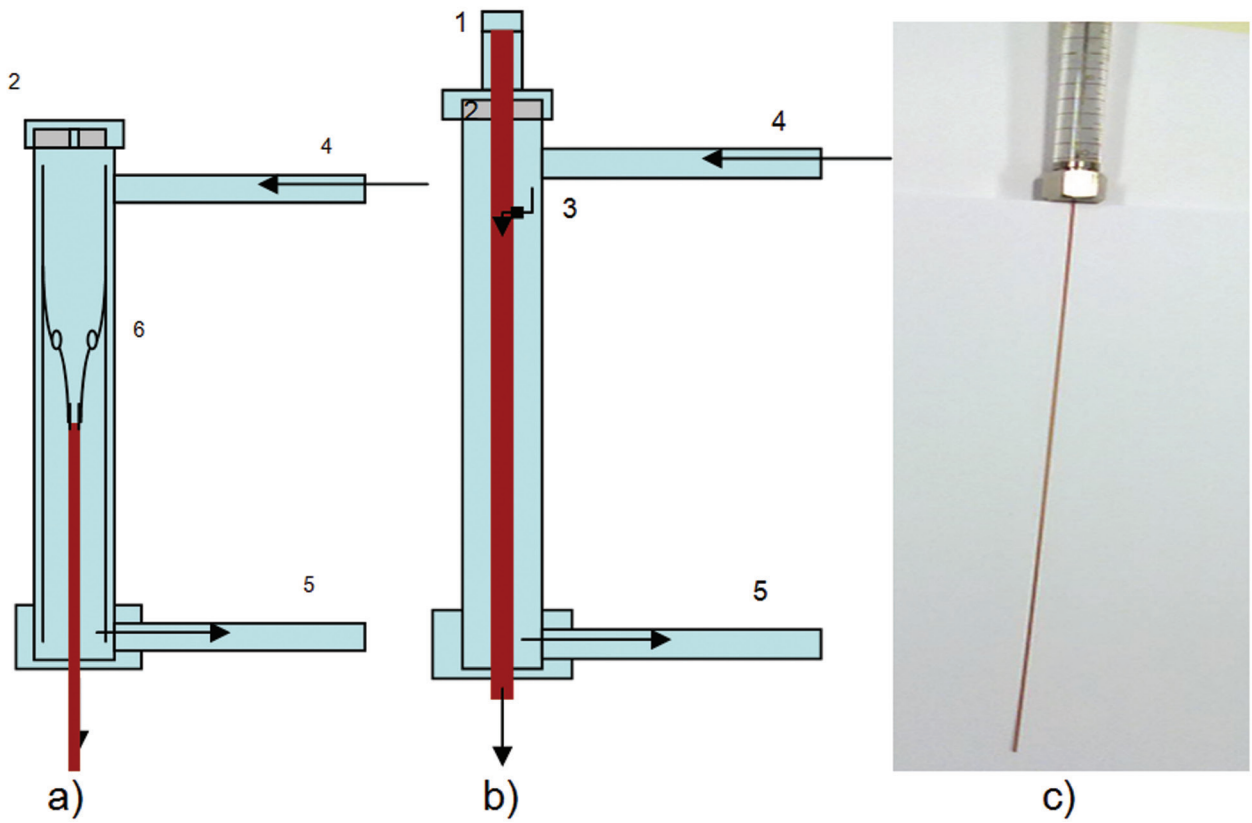

Figure 1. a) On-column injector made with a glass liner consisting in a glass funnel with three small holes (6) to allow the normal split flow. The polyimide free 0.53mm i.d. capillary adjusts to the bottom of the funnel; b) On-column injector made with a 0.53mm capillary to which a partial cut (3) was made at 4 cm of the front end and mounted it trough the septum injector (2). A silicone cap (1) at the top of the capillary maintains the injector under pressure and is removed to allow a convenient syringe capillary needle insertion. Normal split flow passes trough (4) to (5); c) Modified capillary needle syringe (not to scale).

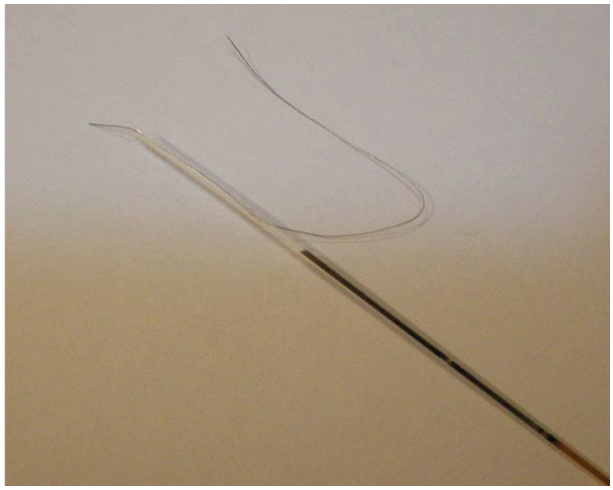

Figure 2. Photograph of one 0.53mm capillary guard column with a cut. To facilitate visualization the outer polyimide layer was burnt and an iron wire was inserted through the hole.

with a liner commercially available, the Uniliner from Restek or the Shimadzu simple on-column solution[4], but with these the normal split flow is not possible. One of the advantages of this method is the easy exchanging to normal split/splitless mode, possible with minimal hardware and settings modification which consist in a simple liner exchange.

The cold on-column injections were successful with this method but a difficulty sometimes arouses in the injection process caused by physical obstruction of the syringe needle in contact with the capillary guard column top end. This occurs due to conflicting alignment of both capillary ends and means that the adequate funnel build-up is difficult to make. Because of this problem we study a potential alternative way to carry out the desired on-column GC injection.

As a second approach to turn possible the needle insertion in the medium bore guard column we have placed the guard column top end, with a silicone cap (see below), in a position above the rubber septa to allow easy needle insertion with the modified capillary syringe referred earlier (Figure 1-b). However the carrier gas must enter the column by some way. The making of a small cut in the 0.53 mm i.d. capillary guard-column at a distance of 4-5 cm from the column front end was the solution (see position 3, Figure 1-b). The cut forms a hole which is positioned inside the injector and allows the normal carrier gas flow trough the column. The "split" flow is as usual adjusted by a valve connected at the base of the injector for adjusting head pressure. This flow is optional and in our case was maintained for removing any reflux that can occur during the injection sequence (see below). This system requires closing and opening the top of the column, for injection purposes. This was easily achieved with a silicone rubber septum which is placed/ removed in the column top end usually by inserting it a few millimetres. The method proved to seal adequately the column top and was fully verified in a number of tests, done with the GC electronic pneumatic control (EPC) head pressure system. The head pressure stability during open/close events was without any problems in the range of head pressures usually found in GC injectors (50-250 KPa).

Since in the injection process the syringe is inserted trough the capillary top from which the cap is removed the carrier flow divides at the capillary hole to outside and to the column, what turns possible the carrier gas/injected sample to back flush. To avoid or diminish this possibility the injection syringe uses a capillary needle with at least 20cm. With such syringe needle we never have found any troubles in chromatogram resolution or loss of sensibility.

The possibility of making a cut in a very brittle and fragile material such as glass or quartz capillary was doubtful at the first glance, but after some training we have worked out a way of doing it, as it can be seen in Figure 2. The cut was made with a high speed silicon carbide abrasive disk slowly approaching the capillary tube. One of the problems was to known if such a delicate spot in the column does not turn it so fragile that it cannot be handled. Since the polyimide coating remains at the cut boundary in ours experience the capillary tube with a hole showed enough stability to allow a high number of syringe insertions, but careful must de draw in handling and inserting the guard column in GC injector for the risk

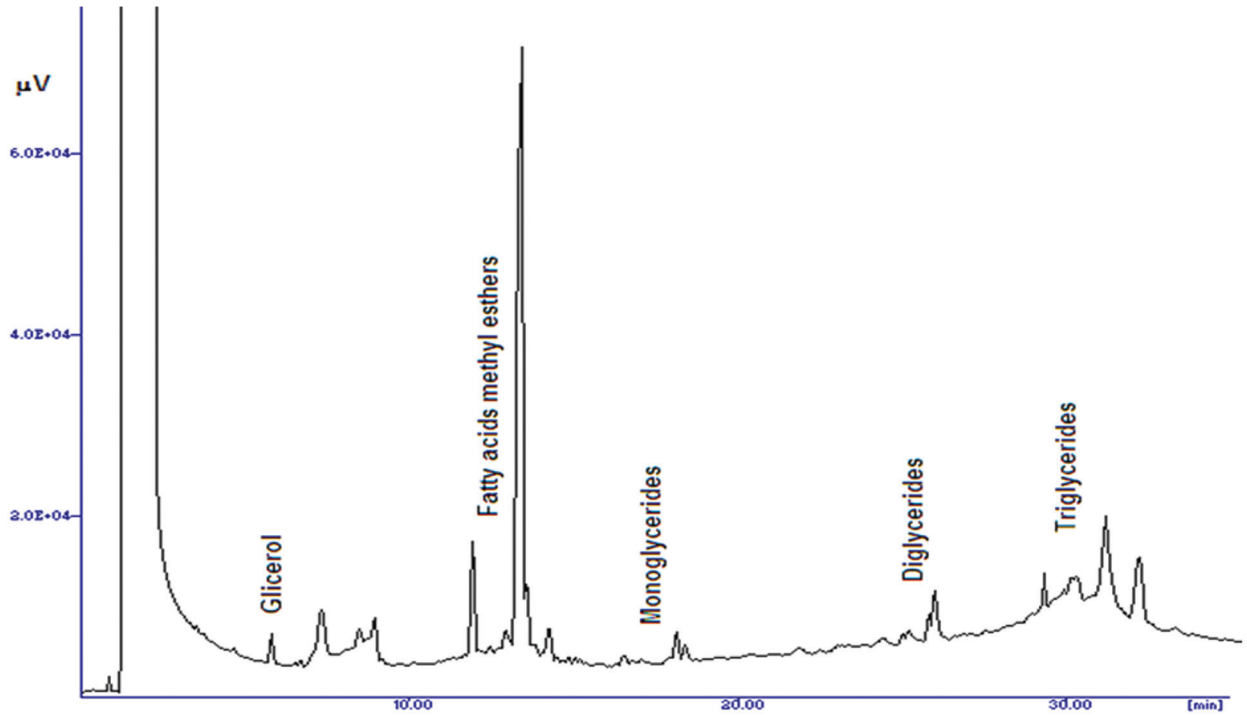

Figure 3. On-column injection of 1µL waste oil biodiesel derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) Conditions: See Experimental section.

of broke. Especially important is not making any bending forces which tend to break the capillary. In future we will try do visualize a way to reinforce the spot, namely by adding polyimide coating to the new cut surfaces. The possibility of making a hole through chemical attack (e.g. fluoridric acid) is another possibility under scrutiny that potentially lead to a not so fragile spot.

Preliminary repeatability studies were made by consecutive injections of hexadecanoate and octadecenoate methyl esters mixture (12% and 88%, v/v; respectively) from synthetic biodiesel sample in the presence of hexadecane as internal standard using the modified funnel liner described above. Peak area relative standard deviation values (%RSD) yielded for hexadecanoate and octadecenoate methyl esters were %RSD = 14.6 and 8.8, respectively (n = 5). Under these conditions a chromatogram example of waste biodiesel analysis is presented in Figure 3. These are good results which show the ability of this solution as a cold on-column injection mode. A more complete study of the precision and repeatability of these injections methods in wax analysis in olive oil are under way and will be published elsewhere.

4. Conclusions

The simple and reversible modification of the normal split/splitless GC injection system was made by two methods allowing the cold on-column injection technique for dedicated methods, e. g. biofuels or wax in olive oil analysis. Both methods allowed a simple and convenient cold on-column sample GC injection. The method using a hole is very simple and was the best preferable choice in our experience.

The possibility of making holes in quartz capillaries was demonstrated and opens news vistas of using them in other analytical techniques, in our opinion it could turn possible an easy sample injection in Capillary Electrophoresis (CE) technique.

In future developments we will try to accesses a method of capillary cut reinforcement trough polyimide coating.

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