An Automated "4 × 4" Sample Applicator

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1 Introduction

In modern trace analysis chromatographic techniques play an important role. To increase the speed of analysis and to decrease costs, automatization of chromatographic methods is being introduced in most laboratories. Gas and liquid chromatographs may be equipped with commercial automatic injection systems and "on-line" data storage and handling.

TLC is essentially an "off-line" technique and not so easily automated. The use of two-dimensional development, which is a necessity in trace analysis, complicates automatization. This disadvantage could, however, be compensated for by carrying out several chromatographic runs at the same time. Therefore, the speed of sample application should be increased.

In an earlier paper a special two-dimensional developing mode called "4 × 4" HPTLC was described [1]. The "4 × 4" mode permits the analysis of four samples simultaneously on one 10 × 10 cm HPTLC plate. Moreover, the use of reference series can be reduced by half.

The "4 × 4" mode can be used with any sample applicator. Optimal use of the "4 × 4" mode is obtained when all four samples are applied simultaneously. This is possible with the prototype of the special sample applicator described previously [1]. However, like its older "single syringe" version [2], this sample applicator is still hand-driven and occupies one member of the staff during the spotting operation, which is a very boring task.

In the present paper a more advanced and automated model of the "4 × 4" spotter is described. With this model, 4 samples of *ca.* 10 µl may be applied unattended as the sample spots on a 10 × 10 cm HPTLC plate in *ca.* 7 minutes.

2 Experimental

2.1 Apparatus and Reagents

The following equipment and apparatus were used: HPTLC plates (10 × 10 cm; *e.g.,* Merck 5547 or 5631); 10-µl syringes (*e.g.,* Hamilton 701, 90° cut needle point style); a linear HPTLC developing chamber (*e.g.,* Camag 28510). A microscope which was a gift from *Prof. Dr. A. De Schrijver* was used. Parts for the drive unit were obtained from a local electronics supply shop.

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2.2 "4 × 4" Sample Auto-Applicator

The "4 × 4" sample auto-applicator consists of two major parts: the applicator itself and the drive unit. The basic element of the applicator was an old microscope. As many parts of this microscope as possible were included in the spotter, with only necessary minor modifications. A schematic of the applicator is given in **Figure 1**. The HPTLC plate is placed on the microscope table. Fixing of the plate is obtained with a minor modification of the fixing mechanism. Using the translation mechanism of this table, the HPTLC plate can be positioned horizontally over a distance of *ca.* 4 × 4 cm. The barrel piece of the microscope was exchanged for a machined, "4 syringe" holder in which four Hamilton 701 syringes could be placed. The needles of the syringes are aligned with respect to the sample application points. These form a square of 8.5 × 8.5 cm. Since the length of the syringe needles may be different the height of the needles has been made individually adjustable by means of four screws. The coarse and fine focusing mechanisms of the microscope body were used for the vertical displacement of the syringe holder. Simultaneous dispensing from the four syringes is produced by the movement of a metal ram actuated by a stepping motor.

For evaporation of the solvent during the application, four special N-jets were constructed. The jets are formed from metal tubes of 3 mm i.d., and blow nitrogen to the center of the forming spot. The

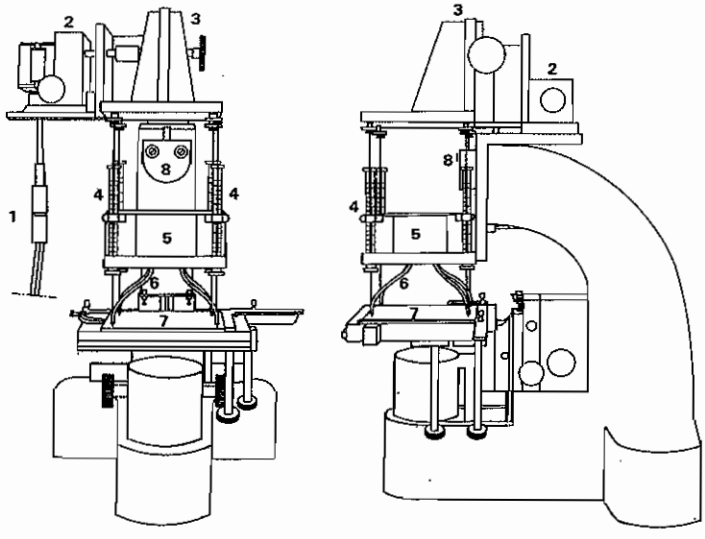

Figure 1

Schematic of the "4 × 4" HPTLC sample auto-applicator: left = front view, right = side view. 1, connection with drive unit; 2, stepping motor; 3, metal ram; 4, syringes; 5, syringe holder; 6, N-jets; 7, HPTLC-plate; and 8, limit switch.

N-jets are aligned in accordance with the sample application sites. A secondary function of these N-jets is exact positioning of the syringe needles.

With this "4 × 4" sample auto-applicator, 4 samples of *ca.* 10 µl are applied simultaneously to one HPTLC plate in *ca.* 7 minutes.

2.3 Drive Unit

The sample applicator is driven by an Airpax 4SQ-0106 stepping motor with 1.8° step angle and coils energized by a Philips SAA1027 integrated circuit. Rotational direction was set as required for forward translation motion of the plungers.

A combination of two 555 timing circuits serve to generate the step input. By connecting the output of the first 555 to the reset input of the second 555, we obtain step pulses that are generated only during the pulse interval established by the first 555.

With this drive system the rate of sample delivery is determined by the step pulse repetition period. A value was chosen which gave a flow rate of 1.5 µl/min. The total amount that is delivered – once the rate has been fixed – is set by the width of the enable pulse. This was programmed as required for delivering 9 µl aliquots.

A microswitch is mounted on the syringe holder as a safety precaution in order to detect the end of travel of the ram and interrupt the power to the stepper motor.

A schematic of the electronic circuit of the drive unit is available from the authors.

3 Results and Discussion

The success of a sample applicator depends on its performance and its ease of use. Only the analysts who work with the apparatus daily are proper judges of a (un)successful design. The prototype of the "4 × 4" sample applicator previously described [1] was not very well accepted for routine lab-work. During its use several shortcomings were discovered. These could only be overcome by designing an entirely new apparatus.

One of the main problems with the first prototype originated from the Hamilton PB-600 dispensers; *i.e.*, it is very difficult to mount a Hamilton syringe into a Hamilton PB-600 dispenser with a reproducible alignment of the syringe with respect to the dispenser and, consequently, 4 syringes *vs* 4 dispensers will always be differently aligned. Therefore, focussing rings had to be mounted on the N-jets, through which the needles of the syringes were forced. This took considerabe time and patience. In addition, during clicking the button of the dispensers, vibrations and force was transmitted to the needles. The needles moved, giving rise to incorrectly matched and bigger spots.

Therefore, we opted for a design without individual dispensers. A syringe holder in which four Hamilton syringes could be mounted directly (with the plungers drawn out) was built. Dispensing from the four syringes is simultaneous and propagated by the movement of a metal ram (actuated by a stepping motor) directly against the plungers.

An unexpected problem was encountered because in some syringes the plunger moves very easily. These syringes start dripping from the moment they are placed in the holder. This problem was solved by sticking a small piece of silicone (*e.g.*, an old septum), with a small hole pierced in it, on the top of the syringe, thus providing additional friction.

The "4 × 4" sample auto-applicator has been tested in our laboratory in regular control analyses of anabolics over the course of one year. From the first moment, this model was accepted with enthusiasm by the laboratory's analysts. It saves a great deal of time and frees personnel from a very boring job. Moreover, sample application is reproducible, and the spots formed are smaller than with the "hand-driven" model. The use of the spotter is of particular advantage when HPLC with fraction collection is used as a clean-up method prior to HPTLC (*e.g.*, analysis of residues of anabolic agents [3]). Instead of one or two fractions (*e.g.*, androgen and estrogen fraction [4]), four to six HPLC fractions have to be applied. We may conclude, therefore, that the use of this apparatus further increases the benefits of the "4 × 4" two-dimensional developing mode.

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