Journal of Chromatography, 92 (1974) 87–93

CHROM. 7254

AN AUTOMATIC CONTINUOUS-FLOW SPOTTING/STRIPING MACHINE

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(Received October 5th, 1973)

SUMMARY

This paper presents the principle, design and application of an automatic continuous-flow spotting/striping machine. The apparatus was specifically designed to apply multiple organic solvent extracts automatically and simultaneously as spots or stripes to chromatography paper. The machine applied twelve 300-μl volumes of a methanol–dichloromethane (4:1) solution to chromatography paper in 30 min as uniform and compact stripes. The dynamic action of a unique mechanical "heart-shaped" cam was responsible for obtaining uniform and compact stripes. A recovery range of 93–98% for [^{3}H]aldosterone (in methanol–dichloromethane) was obtained after pumping the solution through Acidflex® and polyethylene tubing. The remaining [^{3}H]aldosterone was removed by two 100-μl methanol–dichloromethane washes. Urine aldosterone values showed no significant differences when extracts were applied by the machine or manually. This machine reduces the man-hours required for sample applications three-fold and reduces operator tension built up by long hours of tedious manual applications.

INTRODUCTION

Several publications have appeared which describe devices for automatically applying samples to paper[1,2] and thin-layer chromatography plates[3–9]. Most of the machines use micro-syringes or gas pressure to apply the sample. Johnson and Golbey[1] first reported the use of a peristaltic type pump to automatically apply samples to chromatography paper. They reported applying 1 ml of serum extract in 40 min as a spot approximately 13 mm in diameter. Although no recovery values were given, they reported that at least 98% of the solution placed in their apparatus was deposited onto the paper. A similar approach for TLC spot application was later reported by Musil and Fosslein[4].

For our purpose, an automatic spotting machine was unsuitable. Stripes were required to obtain maximum resolution during the chromatographic (paper) step for the determination of urine aldosterone[10]. This publication presents the principle, design, and application of an automatic continuous-flow spotting/striping machine.

APPARATUS

The apparatus is shown in Figs. 1 and 2. Its overall dimensions are 125 cm

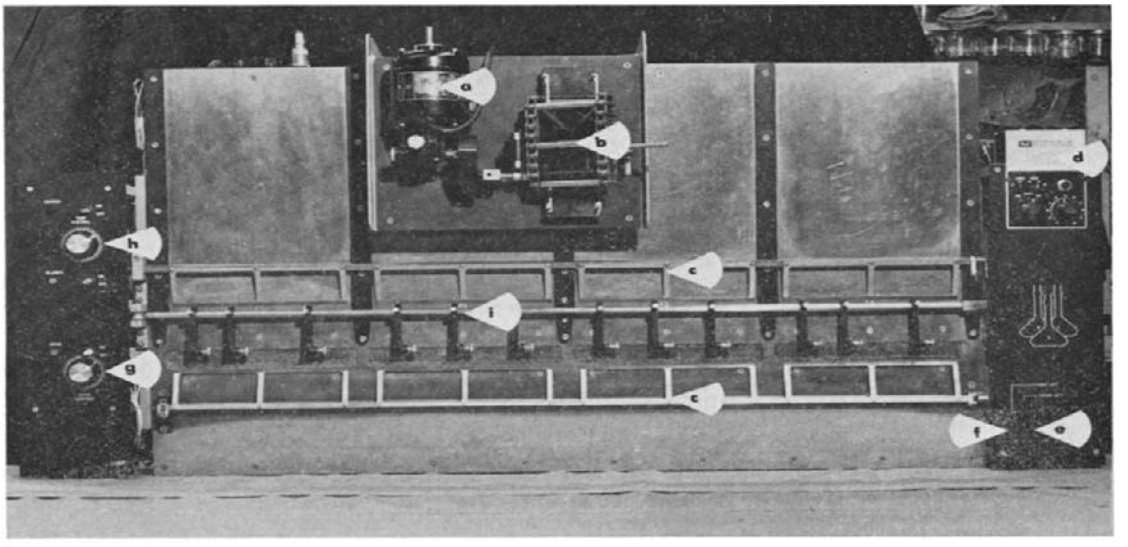

Fig. 1. Top view of spotting/striping machine (without fiberglass cover) showing: (a) pum motor, (b) pump roller chain, (c) paper clamp, (d) motor speed controller, (e) paper clam control lever, (f) sample delivery tubing holder control lever, (g) traverse rod speed contro (h) temperature control, and (i) sample delivery tubing holder and assembly.

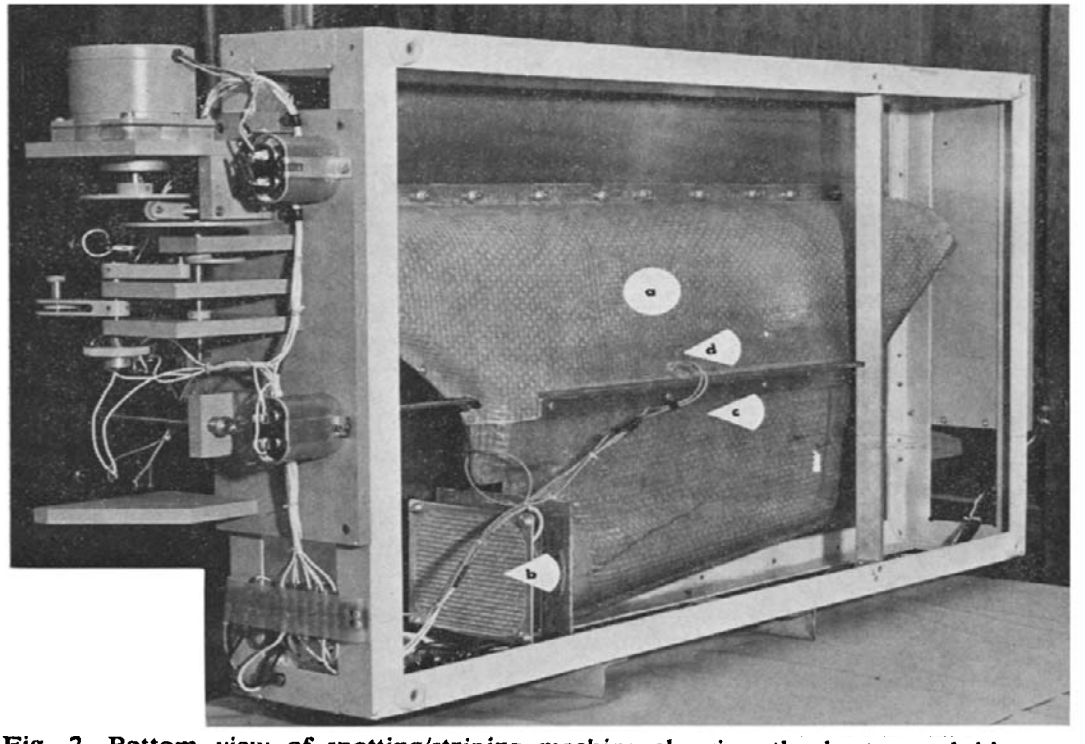

Fig. 2. Bottom view of spotting/striping machine showing the heater and blower systen (a) Fiberglass duct assembly, (b) miniature axial fan, (c) finned stripe heater area, and (d) therm switch area.

long, 60 cm wide and 35 cm high. Details of the machine and its operation are presented below.

Variable speed pump

Mounted on top of the machine is a variable speed peristaltic type pump (modified Technicon Pump I; Technicon, Tarrytown, N.Y., U.S.A.). The roller mechanism and its mounts fit on a flat aluminum plate. A 1/50 H.P. variable-speed motor (Type NSH-12R, Bodine Electric, Chicago, Ill., U.S.A.) is also mounted on the plate. The motor is coupled directly to the roller drive shaft with identical brass gears (4.0 cm diameter, straight-cut teeth). A motor speed controller (Model SL14; Minarik Electric, Los Angeles, Calif., U.S.A.) varies the motor speed. The speed range of the roller mechanism is 0–6 rpm.

Sample transport and delivery tubing

Acidflex® tubing (0.034 in. I.D., 15 cm length), supplied by Technicon, serves as pump manifold tubing and polyethylene nipples (N-9; Technicon) mount the tubing onto end-blocks (Technicon).

Each sample aspiration and delivery tubing (0.034 in. I.D. polyethylene; Technicon) is 32 cm long. A 10-mm length of Acidflex® tubing (0.034 in. I.D.) attaches one end of each tubing section to a nipple (N-9) on opposite ends of a pump manifold tubing.

A 250-μl glass pipet (Microcap; Drummond, Broomall, Pa., U.S.A.) attaches to the sample aspiration tubing by a 10-mm length of Acidflex® tubing (0.034 in. I.D.). The pipet is placed directly into a conical centrifuge tube (17 mm $\times$ 136 mm) containing the urine extract.

Sample delivery tubing holder

Fig. 1 shows the sample delivery tubing holder and assembly. The end of the delivery tubing is inserted through a stainless-steel needle (14 gauge, 5 cm length) till it extends approximately 1 cm beyond the end of the needle. A 1-cm length of Acidflex® tubing positions the delivery tubing in the needle, and the needle is held in position by the holder. The needle holder has slots and clamps down on the needle with the slotted washer. The knurled nut tightens the assembly and holds the needle in the desired position. The holder is held in position on the traverse rod by the knurled knob.

Traverse rod and "heart-shaped" cam assembly

Fig. 1 shows the traverse rod (stainless steel) which supports the sample delivery tubing holder. The rod is 90 cm long and 1 cm in diameter. The rod is supported by rollers and can be rotated upward manually by a series of lever arms connected to a control lever. The rod is connected to the "heart-shaped" cam anodized aluminum) by linkage which can be adjusted to shorten or lengthen the stroke of the rod (Fig. 3). The cam is driven by rotating discs (anodized aluminum) interconnected by an idler wheel; the discs are connected to a drive motor (SLO-SYN®, Type SS50; Superior Electric, Bristol, Conn., U.S.A.). Moving the idler wheel toward or away from the center of the drive disc with the speed control knob will increase or decrease the speed of the traverse rod, respectively.

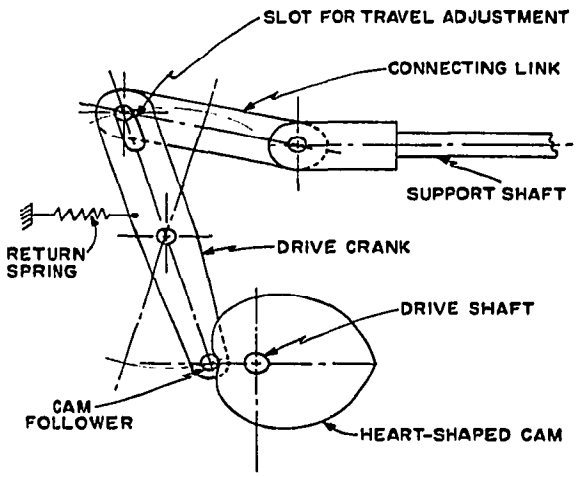

Fig. 3. Mechanical drawing of "heart-shaped" cam and linkage.

Chromatography paper clamps

Fig. 1 shows clamps (stainless steel) which are used to hold the chromatography papers (maximum size, 21 cm wide and 54 cm long) in position. The clamps are simultaneously raised and lowered mechanically with a control level.

Heater and blower system

Fig. 2 shows the heater and blower system. The finned strip heater (Cat. No. 2A810F102; General Electric, Los Angeles, Calif., U.S.A.) is automatically controlled by a thermoswitch (Cat. No. 1700; Fenwal, Ashland, Mass., U.S.A.). The blower system consists of a miniature axial fan (Model 1000A; available from Newark Electronics, Inglewood, Calif., U.S.A.), which blows air across the heater. The heated air enters a duct (fiberglass) which distributes the air to four slots (2.5 cm wide and 22 cm long) directly beneath the application zone of the chromatography paper (Fig. 1). Baffles are located in the air duct to provide even distribution of heated air at each exit slot. The temperature of the air was routinely heated to 45° (at exit slot); air can be safely heated up to 80° with this system.

Instrument operation and automatic sample application

The chromatography papers are positioned, the fan and heat are turned on, the delivery tips are positioned and the traverse rod is turned on. The glass sample aspiration tubes are placed into the conical centrifuge tubes and the extracts are pumped into the glass tubes. The centrifuge tubes are rinsed with 100 μl methanol–dichloromethane (4: 1) and the washes are pumped into the glass aspiration tubes. Sufficient lengths of polyethylene tubing are used to accommodate the extract and two solvent washes.

The pump is operated at maximum speed and the solutions are pumped to the delivery tips. The motor controller is then set to provide a roller chain speed at 6 rph. The machine will now automatically apply each extract and both washes as uniform stripes in approximately 30 min.

RESULTS AND DISCUSSION

The most important consideration of the automatic spotting/striping machine was the selection of suitable pump tubing. Experiments were conducted which showed that the dichloromethane solvent would dissolve Tygon® and Solvaflex® tubing. The Acidflex® tubing was selected since it was only slightly affected by the solvent In routine use the Acidflex® tubing began to degenerate after two months of continuous use. At that time some of its elastic properties diminished and the rubber began to dissolve. When this occurred the polyethylene tubing leading from the pump was coated on the inside with a black residue.

Urine extracts in 100 μl methanol–dichloromethane and two 100-μl methanol–dichloromethane washes for each extract were applied by the automatic striping machine to Whatman No. 1 chromatography paper. Table I shows the dimensions of the fluorescent stripes, which were measured under long-wave UV light. The results show that uniform stripes (12–16 mm long and 4–7 mm wide) for 300 μl methanol–dichloromethane solution could be obtained with the instrument. These uniform stripes were necessary to obtain satisfactory chromatographic separation of urine aldosterone from other urinary constituents. Because of this consideration the spotting machine of Johnson and Golbey[1] was unsuitable for automatic sample applications. Narrow uniform stripes, which showed no "dumb-bell" effects, were produced by moving the sample tip back and forth over the paper along 1.5 cm while continuously dispensing the sample. "Dumb-bell"-shaped stripes were eliminated by using a "heart-shaped" cam to minimize lag time at the end of each stroke of the traverse rod to less than 1 sec.

TABLE I
STRIPE SIZES FOR URINE EXTRACTS AND WASHES

Extract No.	*Length (mm)*	*Width (mm)*
1	16	6
2	16	6
3	16	7
4	15	5
5	14	4
6	12	4
7	12	5
8	13	5
9	13	5
10	13	5
11	14	5
12	15	5
13	14	4
14	14	6
15	14	4
Average	14	5
Range	12–16	4–7

TABLE II

RECOVERY OF [^{3}H]ALDOSTERONE STANDARD

Sample	*Counts/min*	*Recovery (%)*
Before pumping	150,000	—
After pumping		
1	146,000	97
2	139,000	93
3	145,000	97
4	146,000	97
5	147,000	98
6	143,000	95
7	147,000	98
8	144,000	96
9	146,000	97
Average	145,000	96
Range	139,000–147,000	93–98

TABLE III

URINE ALDOSTERONE VALUES — AUTOMATIC MACHINE *vs.* MANUAL APPLICATIONS

Application method		*High pooled urine (μg/24 h)*	*Low pooled urine (μg/24 h)*
(A) Manual	Range	28–42	7–11
B) Automatic			
1		30	8
2		25	8
3		44	5
4		41	7
5		43	9
6		40 *	8
7		35 *	8
8		33 *	11
9			12
10			11
11			11 *
12			12 *
13			13 *
Range		25–43	5–13

* Denotes one 100-μl wash of methanol–dichloromethane instead of two.

100-μl aliquots of a methanol–dichloromethane solution containing 150,000 cpm of [^{3}H]aldosterone were pumped into vials of scintillation reagent. The radioactivity was then measured by a Packard Liquid Scintillation Counter (Packard,

Downers Grove, Ill., U.S.A.) (Table II). Recovery studies show that 96% of the [^{3}H]aldosterone could be recovered without any wash. This indicates little interaction between the isotope and the tubing system (polyethylene or Acidflex®). The two washes removed the remaining radioactivity.

Table III presents values for urine aldosterone (high and low pools) using the automatic striping machine compared with values obtained using manual applications. Values for pooled urine containing high and low aldosterone concentrations show good agreement when the extracts were applied by either the automatic machine or manually.

One technician can operate three machines daily and apply approximately 150 extracts per day (36 samples at a time). These machines reduce the man-hours required for applications from 24 h to 8 h. Equally important as the time-saving benefit, operator tension built up by long hours of manual sample applications is also significantly lowered.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the invaluable technical advice of Dr. Ralph E. Thiers. The instrument design and engineering by Mr. John Felder, and instrument fabrication by Mr. George Branch are also greatly appreciated.

REFERENCES

1 B. Johnson and M. J. Golbey, *J. Med. Lab. Technol.*, 26 (1969) 55.
2 C. B. Mankinen and D. Fischer, *J. Chromatogr.*, 36 (1968) 112.
3 C. B. Mankinen and R. M. Sachs, *J. Chromatogr.*, 47 (1970) 509.
4 F. Musil and E. Fosslien, *J. Chromatogr.*, 47 (1970) 116.
5 J. S. Chahl and C. C. Kratzing, *Clin. Chim. Acta*, 26 (1966) 177.
6 E. Stahl and E. Dumont, *J. Chromatogr.*, 39 (1969) 157.
7 S. Samuels, *J. Chromatogr.*, 32 (1968) 751.
8 T. Darocha, C. H. Gray and R. V. Quincey, *J. Chromatogr.*, 27 (1967) 497.
9 F. A. Vandenheuvel, *J. Chromatogr.*, 25 (1966) 102.
10 B. Kliman and R. E. Peterson, *J. Biol. Chem.*, 235 (1960) 1639.