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A semi-automatic device for multiple sample application to thin-layer chromatography plates

One-dimensional chromatography of amino acids in urine has become established as a standard technique for the screening of metabolic disorders of the newborn. Filter paper was the separation medium favoured by the earlier workers in this field[1-3], but paper has two considerable drawbacks: that a prolonged period of solvent migration is necessary — at least 16 h for adequate separation of amino acids — and, secondly, that because of the coarse texture of the paper, the separated amino acids tend to diffuse and to overlap.

To overcome these defects, thin-layer chromatography (TLC) has been used, which offers the advantages of reduced development time, a homogeneous medium giving improved separation, and the possibility of varying the separating medium. However, again TLC has its disadvantages — one being a lower sample capacity than paper, another being the technical expertise necessary to coat the plates reproducibly by hand; as the commercially available precoated plates are prohibitively expensive for large scale screening operations. In the latter regard automated coating devices are available*, which for large scale operations reduce the cost to a level comparable to the use of filter paper. Also the ancillary equipment required is much simpler and less expensive for TLC than for paper. However, because of the relatively small fluid capacity of the TLC plates, the application of samples remains a time-consuming procedure. Several companies have introduced automatic spotting devices designed to deliver pre-determined quantities

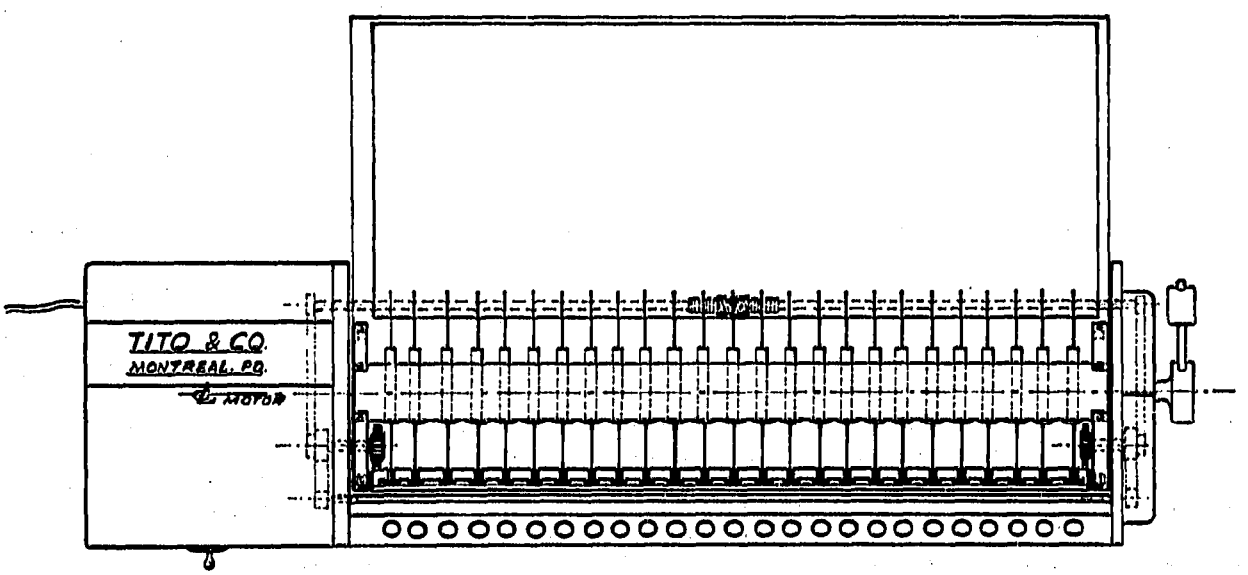

Fig. 1a.

* TLC Spreader No. 21652, Camag, Muttenz, Switserland.

Fig. 1b.

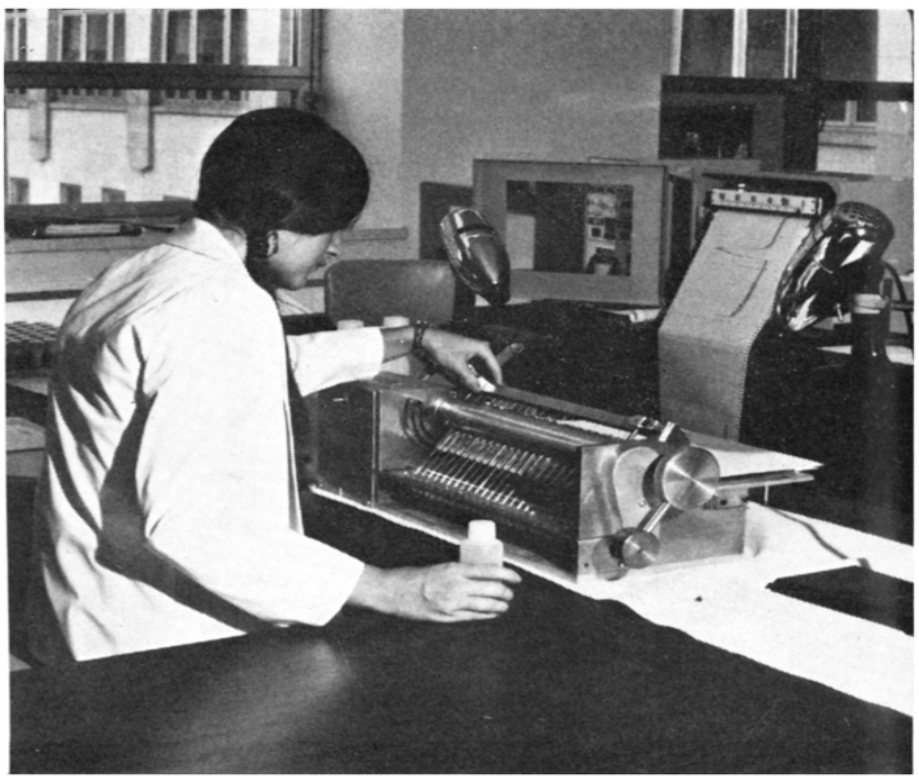

Fig. 1c.

Fig. 1. (a) Diagramatic plan view representation of the device (to scale), (b) Rear view of device, (c) Front view of device.

of samples to plates*, but these are designed to apply aliquots of the same sample in a highly reproducible fashion and not to deliver simultaneously a large number of different samples.

In setting up our urine screening programme[4], which currently involves approximately one hundred thousand samples a year for one way amino acid chromatography with future application to organic and keto acids, the time involved in the manual application of three hundred samples daily to TLC plates proved prohibitive and so a semi-automatic spotting machine was designed by the authors and constructed by Tool and Die Precision Works, Montreal. The machine was designed to aspirate from twenty-five samples (in practice, twenty samples and three standards) and to apply the sample as 1-cm streaks to a 20 × 40 cm thin-layer plate.

Description

The device (Fig. 1), which is fabricated from stainless steel, consists of a horizontally mounted rod carrying twenty- five Hamilton® syringes with the plungers of the syringes held by a steel bar which is attached at its ends to two threaded strips. At the rear of the device the TLC plate is held on a horizontal plate which is driven laterally for 1 cm by a threaded rod. The plate is adjustable to give a clearance of about 1 mm when the rod is turned fully clockwise. Power for the device is provided by a 1/8 hp electric motor and the samples are contained in Technicon® micro cups held in a detachable Plexiglas® support at the front of the device.

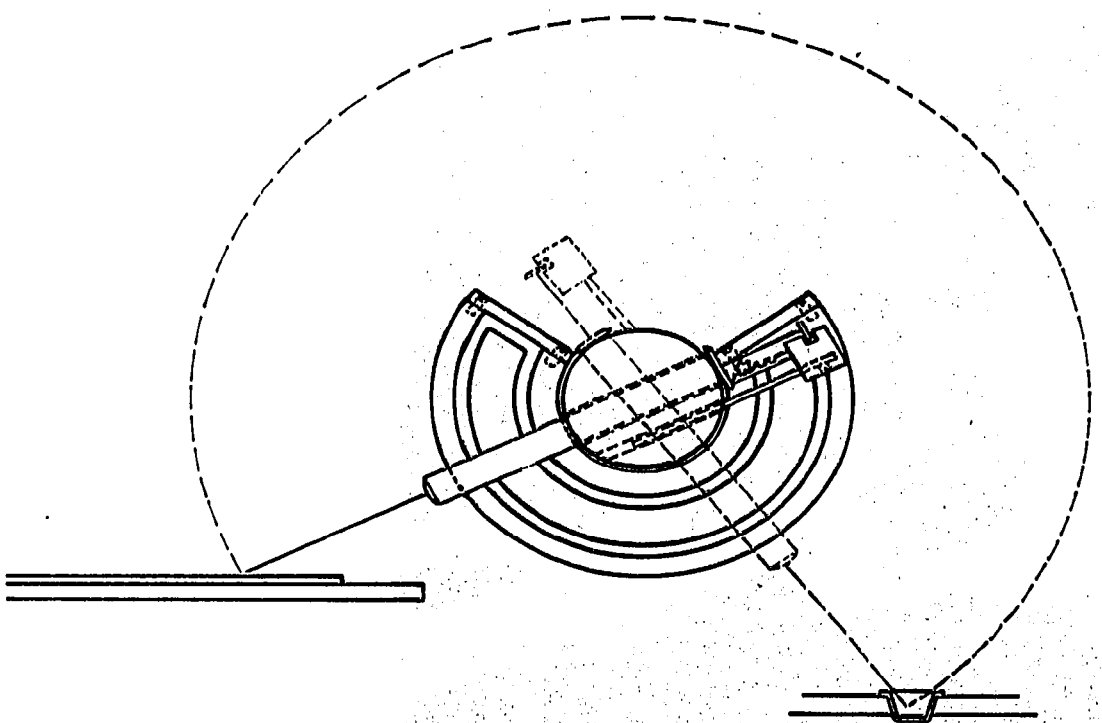

Fig. 2. Movement of syringes during filling and delivery, sample cups to the right.

* Desage Autoliner, Brinkmann Instruments Inc., Westbury, N. Y., U.S.A. Sample Streaker, Shandon Scientific Company Inc., Sawickley, Pa., U.S.A. Rodder Streaker, Rodder Instrument Co., Los Altos, Calif., U.S.A.

Operation

With the handle the rod is rotated counterclockwise (Fig. 2) bringing the tips of the syringes below the fluid level in the sample cups. The syringes are now filled by pulling the plunger retaining bar, and the rod is rotated fully clockwise engaging the thread of the steel strips with the gear wheels. The motor is switched on and the plungers are driven in while the TLC plate moves laterally simultaneously. After delivery is completed, the TLC plate is removed and the syringes are emptied manually by depressing the plunger-retaining bar fully, wiping the fluid from the tips of the needles with a cellulose tissue and the TLC retaining plate is disengaged and returned manually to its starting position. The device aspirates four fifths of the capacity of the syringes but only delivers one fifth; so as the syringes are filled with fresh samples, the fluid in the needles from the previous sample will not contaminate the current sample and so it is not necessary to wash the syringes between samples.

Results

The patterns obtained are reproducible (Fig. 3) without overlap, and six hundred samples may be processed in 1 h.

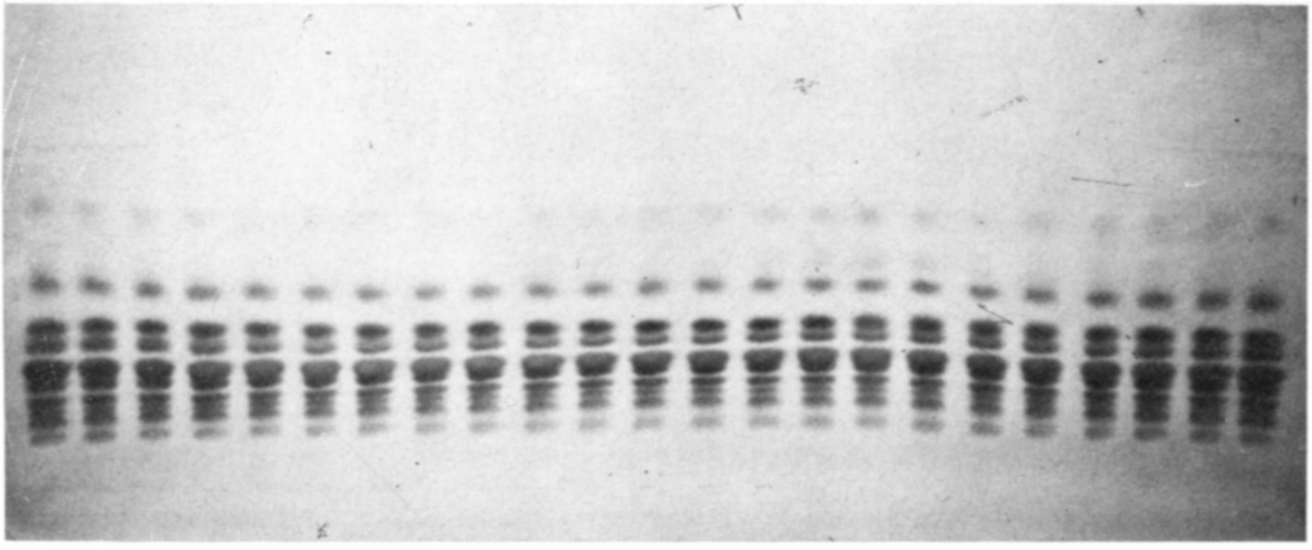

Fig. 3. Amino acid solution showing separation and reproducibility of pattern.

In practice the two syringes at the extremities of the rod are not used as solvent migration is uneven at the edges of the TLC plate. As the samples we were using were, in fact, diluted urine; it was necessary to make three separate applications of the same sample which can be accomplished in 1.5 min using this apparatus*. To speed sample drying when multiple applications are made of the same samples, a hot air blower is positioned at each side of the TLC plate but this is not necessary if single applications are made.

The apparatus was designed to function in as simple and economical a fashion as possible and could be improved at increased cost, *e.g.* the provision of a

* The apparatus may be purchased from TITO and Co.

heated TLC plate support and the basic spotter can be modified for specific applications.

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