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DETERMINATION OF CREATININE IN SOUPS AND SOUP PREPARATIONS BY ION-EXCHANGE CHROMATOGRAPHY

II. AUTOMATIC APPARATUS

A. CARISANO, M. RIVA AND A. BONECCHI
Research Laboratories of Star Food Co. Ltd., 20041 Agrate Brianza (Italy)
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SUMMARY

An automatic apparatus for the determination of creatinine in soups and soup preparations by cation-exchange chromatography and the continuous UV spectrophotometric monitoring of the effluent is described. The data obtained in the analysis of a soup preparation for sixty consecutive days showed a small scatter. The recovery of creatinine amounted to 99.52 ± 1.80% of the most probable value. The main features of the apparatus are described in detail.

INTRODUCTION

A previous article[1] dealt with a method for the determination of creatinine in soups and soup preparations by separating this compound from interfering substances on a column packed with a cation-exchange resin and by subjecting the effluent to continuous UV spectrophotometric analysis. This was a discontinuous method because all the operations in it, including the sample introduction, were carried out manually. This method has now been fully automated, and the apparatus developed is described below.

APPARATUS

The automatic analyser shown in Fig. 1 is composed of the following main parts:

(I) 4-l bottle A containing 0.4 *M* sodium acetate buffer (pH 4.35 ± 0.01);
(II) pump P for passing the buffer solution to column C at a rate of 77 ml/h;
(III) column C packed with a cation-exchange resin;
(IV) a sample-introducing device (patent pending) connected to column C;
(V) thermostat (not shown) which keeps the water circulating in the water jacket of the column at 56.5°;
(VI) UV spectrophotometer linearized between 0 and 1000 optical density units, fitted with photomultiplier and a 300-μl quartz flow cell;

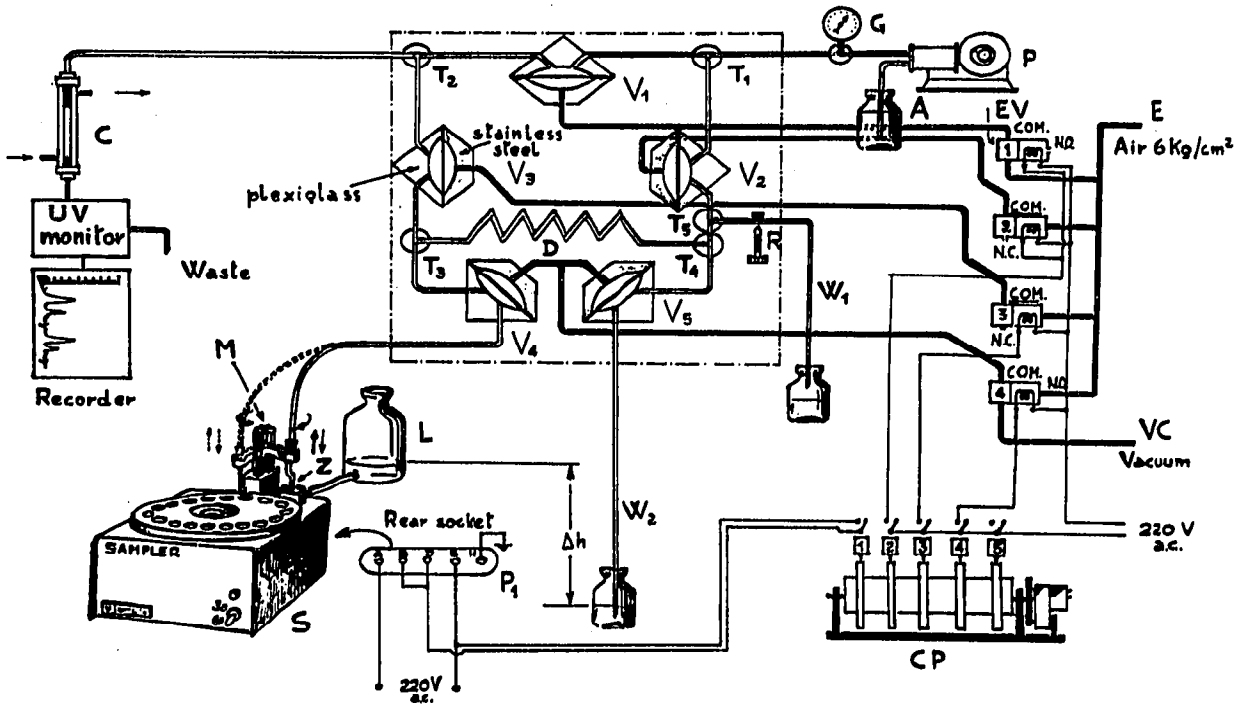

Fig. 1. Scheme of the automated analytical system for the determination of creatinine. For key, see text.

(VII) potentiometric recorder with a suitable sensitivity and a response time of about 1 sec (full scale). (For easy measurement of the peak areas, the deflection should be at least 25 cm.)

Fig. 2 shows the chromatographic column C with all its accessories. This column has an I.D. of 0.57 cm, an O.D. of 1.03 cm, and a total length of 22 cm. The resin (Aminex A_5*; heigt 11 cm) is kept firmly in place by a small PVC piston at the top and bottom. The end of each piston is fitted with a porous teflon disk (pore size 8 μ), which permits the eluent to flow through, while retaining the resin particles. The lower piston is supported by a ferrule while the upper piston can move, since the ferrule contains a spring, exerting a pressure of at least 7 kg/cm² on the piston head. The tightness of the pistons against the walls of the glass column is ensured by silicone rubber O-rings. Polyethylene tubing (I.D. 1 mm; O.D. 2 mm) is used for all connections, namely the pneumatic connections for the actuation of the diaphragm valves and the tubes carrying the buffer.

Sample-introduction device

Fig. 1 shows that the sampling system, whose parts are enclosed by a dash–dot line, is between pump P and the chromatographic column C. Other parts shown are as follows: (1) eluent reservoir A; (2) washing liquid reservoir L; (3) sample dispenser S; (4) compressed air inlet (6 kg/cm²) E; (5) five small diaphragm valves V_1–V_5 actuated by compressed air; (6) 4 pneumatic electro-valves EV_1–EV_4, which directly control the diaphragm valves; (7) cyclic programmer with a rotating drum CP, actuating the electro-valves EV_1–EV_4; (8) water pump VC generating vacuum. The whole system is

* Bio-Rad Labs., Richmond, Calif., U.S.A.

controlled by the programmer via microswitches 1–4, connected to the valves EV and to sample dispenser S. A more detailed picture of the part inside the dash–dot line also includes the following components: (1) metering channel D (0.5 ml); (2) three-way joints T_1–T_5; (3) micrometric screw clamp R on the discharge tube W_1. The micro-valves, the sample dispenser, and the programmer will be described before discussing the operation of the system.

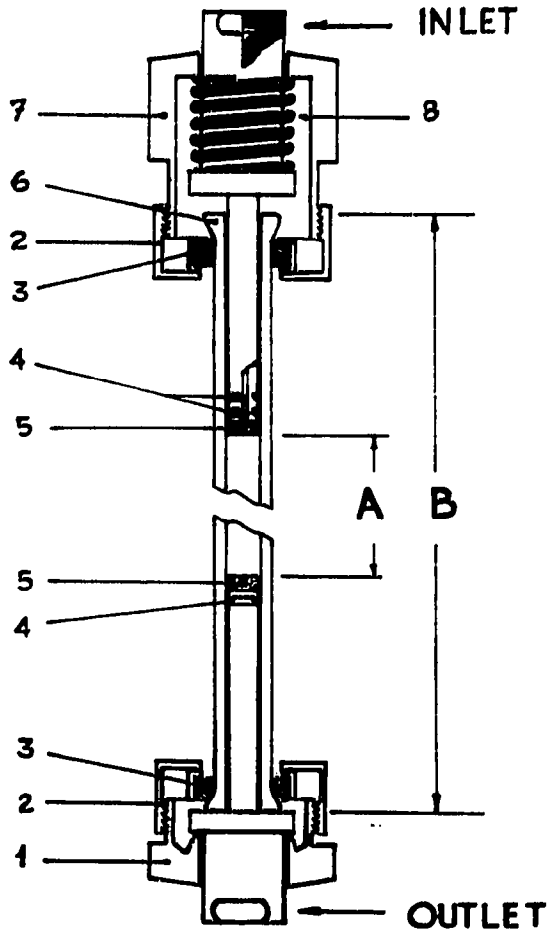

Fig. 2. Chromatographic column and its accessories (A = 11 cm; B = 22 cm). (1) Male ferrule made of PVC; (2) Female ferrule made of stainless-steel; (3) Teflon washer; (4) Silicone rubber O-rings; (5) Porous teflon disks (thickness 3 mm); (6) Glass column; (7) Male ferrule made of PVC, housing the spring; (8) Spring.

Diaphragm micro-valves

The diaphragm micro-valves deserve a brief description, although they have been used before in various automatic devices. They score because there is no mechanical friction here between the moving parts that effect closure, the dead space is negligible, and the operation is rapid, reliable, and easy to actuate by compressed air, regulated by electro-valves. As can be seen in Fig. 1, each diaphragm valve consists of a lens-shaped cavity, divided along the longer axis by an elastic membrane (coupled silicone and Viton). This membrane ensures perfect insulation between the analytical circuit and the control circuit. The transparent plexiglass top of the valve houses the

tubes for the liquid, while the stainless-steel bottom houses the membrane-command tube. When this part is pressurized, the membrane is deformed, and the flow of the liquid is stopped. When the pressure is discontinued and/or partial vacuum is created, the membrane is deformed in the opposite direction, and the valve is kept open. Four units with two independent command circuits are housed in a block, and the connection of two such blocks gives the five functions needed for operating the system shown in Fig. 1. The two blocks in our apparatus were made by Carlo Erba* after our design.

Sample dispenser

The sample dispenser device S (type CLA 1510), made by Carlo Erba, essentially consists of a rotating disk that can accomodate 40 sample holders. A mobile device M is mounted at a fixed point over the disk. This device introduces a suction tube into the sample holders. This operation alternates with the introduction of the same tube into the washing liquid reservoir at point Z. A positioning motor and a small timing motor perform the operations in the required sequence. The independent cyclic arrangement of the commercial apparatus was modified; a momentary closure of switch 1 of the general programmer (Fig. 1) now starts the process, while the end is brought about automatically by the positioning motor as soon as the suction tube is introduced into the washing liquid (point Z). The modification was done in accordance with the circuit shown in Fig. 1, and the power supply (220-V a.c.) is now introduced not in the normal cable (which remains unused) but through connector P_1 on the back of the sample dispenser. This modification is essential to ensure the synchronized operation of the various parts.

Programmer

The programmer is of the drum type and is fitted with a motor and a reduction gear that perform one complete revolution every 35 min. The command sections of the microswitsches 1–5 are made of a moulded plastic and can be easily replaced. It is therefore possible to set up any program for each channel (the phase during the calibration being then varied). Four channels were used in the apparatus described here. The programmer was chosen from the Carlo Erba's fractomatic range of process-control gas chromatographs.

Operation

Pump P passes the eluent from bottle A into column C via devices T_1, V_1 and T_2. The eluate leaving the column is subjected to continuous UV spectrophotometric monitoring at 240 mμ and is then discarded. Therefore diaphragm valve V_1 is open while diaphragm valves V_2 and V_3 are closed. Furthermore, the sample to be introduced is already in the metering channel D, and hence V_4 and V_5 are closed as well. The suction tube is placed into the washing liquid L at point Z. This is the condition that prevails for the longer time during each analytical cycle; therefore the pneumatic connections between valves V_1– V_5 and electro-valves EV_1–EV_4 are so arranged as to obtain the required functions with all the solenoids non-activated. The microswitches 1–4 of the programmer are open, and the sampler is therefore not energized.

* Carlo Erba S.p.a., Scientific Apparatus Division, 20090 Rodano, Italy.

When the sample is to be introduced, the programmer closes the micro-switch 2, and valves EV_1 and EV_2 are simultaneously energized, which makes valve V_1 close and valve V_2 open. The eluent is discharged into tube W_1 via the constriction R, which is regulated to keep the route $P–T_1–V_2–T_5–W_1$ under a pressure of 1.5 kg/cm^2. The closure of V_1 decreases the pressure and the flow rate in the column, and these changes favour the imminent reception of the sample. After some seconds, the programmer closes micro-switches 3; EV_3 is energized, and micro-valve V_3 opens to permit the sample to enter the column. The sample is pushed along by the eluent, part of which is split off at W_1 in order to keep the pressure and the rate of introduction low.

When the sample has been transferred into the column, the programmer de-energizes valve EV_3, which makes valve V_3 close. After a few seconds, micro-switch 2 opens, and both EV_1 and EV_2 are de-energized, whereby the initial state, with V_1 open and V_2 closed, is re-established. The analytical phase then begins.

During this phase, the line of the metering channel D is rinsed, and then the next sample is fed into it. This sample is introduced into the column when the previous analytical phase is over. In this way, the programmer closes the micro-switch 4, thus energizing valve EV_4. Valves V_4 and V_5 then open on account of a lack of command pressure. To ensure a perfect opening of the inlet and outlet, the membrane is brought back with the aid of vacuum. Owing to the level difference between the washing liquid in the reservoir L and the discharge point W_2, the washing liquid starts flowing spontaneously at this moment along the route $Z–V_4–T_3–D–T_4–V_5–W_2$. Simultaneously with the closure of the micro-switch 4, the programmer closes micro-switch 1, and the sample dispenser is thus energized for 80 sec. The mobile attachment then removes the suction tube from the washing position and places it into a sample holder on the disk. A small amount of air is sucked in during the transit, and this air plug serves to separate the washing liquid from the sample. Thus begins the spontaneous flow of the sample which lasts for 40 sec and fills up the whole system of the metering channel D (part is discarded into W_2). Before the sample (*ca.* 3 ml) in the holder is exhausted, the programmer de-energizes valve EV_4, thus causing V_4 and V_5 to close. The sample is thus detained in the metering channel D. As described before, the internal mechanism of the sample dispenser stops the operation of the mobile suction unit when this is inserted into the washing liquid and when the sample-holder disk advances by one step, offering the next sample for suction.

To stop the whole operation, a dummy sample holder is introduced into the disk after the last sample. On starting the suction phase at this dummy sample holder, the mobile unit closes a low-voltage circuit which is connected to an auxiliary relay and can terminate the power supply to the whole system or can put it in stand-by position. Table I shows the operative positions of the four programmer-controlled micro-switches, and the state of the four electro-valves, which regulate the five micro-valves $V_1–V_5$ of the analytical circuit.

RESULTS AND DISCUSSION

In view of the geometry of the chromatographic system (which is optimized for the separation of creatinine from interfering substances) and in view of the fact that at least 0.5-ml samples are needed for accurate automatic sampling, the sample must be introduced slowly and at a low pressure, as in manual operation with open columns.

TABLE I

ROUTINE SCHEDULE

O = Open; C = Closed.

Time (sec)	*Micro-switch on programmer*				*Micro-valve*				
	1	*2*	*3*	*4*	V_1	V_2	V_3	V_4	V_5
0	O	C	O	O	C	O	C	C	C
10	O	C	C	O	C	O	O	C	C
208	O	C	O	O	C	O	C	C	C
222	O	O	O	O	O	C	C	C	C
1905	C	O	O	O	O	C	C	C	C
1912	C	O	O	C	O	C	C	O	O
1985	O	O	O	C	O	C	C	O	O
2002	O	O	O	O	O	C	C	C	C
2100	O	C	O	O	C	O	C	C	C

In this way, the sample is deposited correctly on the first theoretical plate of the column, and the maximum resolving power is thus ensured. The system described above satisfies these requirements in a cyclic and automatic manner.

Special care was taken to make the micro-valve V_1 close and the micro-valve V_2 open some seconds before micro-valve V_3, thus lowering the analytical-circuit pressure of 4–5 kg/cm² by at least 1 kg/cm² in the V_1–T_2–C section. The latter is then temporarily isolated from the pump and is connected only to the discharge route. The pressure in the section P–T_1–V_2–T_5 is reduced to 1.5 kg/cm², since at this pressure the eluent overcomes the calibrated resistance R and is discharged along W_1. It is only at this moment that the delayed opening of V_3 permits the slow introduction of the sample, waiting in the T_4–D–T_3 section, onto the first theoretical plate of the column. After a sufficient time, microvalve V_3 closes a few seconds before micro-valve V_1 opens, and thus the pressure in the column C decreases from 1.5 kg/cm² to zero.

The conditions of manual sample introduction were carefully reproduced in this manner because distorted and ill-resolved peaks had been obtained in preliminary experiments with the automatic system when the samples were introduced into the column fast and at the usual (high) pressure prevailing in the system. Such sample introduction evidently causes a marked reduction in the efficiency and the resolving power of the column.

To promote an even better collection of the sample on the first plate, the sample was dissolved in a buffer more acidic than the eluent. We used for this 1 m*M* solution of cytosine in 0.3 *M* acetic acid, adjusted to pH 3.70 with 40% NaOH (used previously in the manual method). The sample was prepared and treated exactly as before[1] and then it was dissolved in exactly 5 ml of the 1 m*M* cytosine solution. Whenever necessary the sample was filtered through a Whatman No. 42 paper, an aliquot was placed in a *ca.* 3-ml plastic holder, and the latter was introduced into the sample holder disk.

In this fully automated system, the samples prepared during the day can be analyzed during the night. 24 samples can be analyzed between 6 p.m. and 8 a.m.

Since an internal standard (cytosine) is used, the creatinine values can be found from the ratio between the creatinine peak area (or height) and the cytosine peak area (or height). The evaporation of the sample does not influence this ratio, so the samples can be left uncovered on the plate. The filtration of the samples before the analysis

removes mucilaginous substances which may clog up the column, and so the resin can be used for several months without regeneration. When eventually a pressure of 5.5–6 kg/cm^2 on the gauge G (see Fig. 1) is needed to make the eluent flow, the column is dismantled, and the resin is poured into a beaker and regenerated. This is done by washing it in turn with hot 6 *N* HNO_3, distilled water, hot 4 *N* HCl, and 2 *N* NaOH. The resin, thus obtained in the Na^+ form, is washed repeatedly with distilled water and then replaced into the column, ready for further analysis. When analyzing an average of 12 samples a day, we used a column for about three months before regenerating the resin. The automatic system had the same precision and accuracy as the manual system. The reproducibility of the results was excellent. The creatinine content of a soup preparation was found to be 0.51–0.57% (mean 0.537%) by HADORN's method[2,3], and 0.54–0.57% (mean 0.552%) by the automated method, these analyses being carried out simultaneously for 60 consecutive days. On the basis of the data for the meat extract used, the most probable creatinine content of this soup preparation was 0.555%.

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