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Note

Simple programmable controller allowing the timed collection of fractions in high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) is primarily used in conjunction with on-line detection and quantitation. However, for some applications, for example, the separation and analysis of bioactive peptides from tissue extracts, on-line detection is not always possible because the compounds of interest are either below the limit of detection or are masked by co-eluting material. In such cases, fixed volume fractions of the column effluent can be collected and the substance quantitated using a specific radioimmunoassy (RIA)[1–3] or bioassay[4].

Retention times in reversed-phase HPLC are highly reproducible[1–4] over a wide concentration range. Therefore when the retention time of a standard compound is known, it is possible to collect the portion of column effluent in which it is eluted as a single fraction prior to quantitation.

We describe here a cheap programmable device for controlling a fraction collector which will allow the automatic collection of fractions of varying volume at specified time intervals with a resultant reduction in the number of samples required for assay. The device can be interfaced with an automatic sampler to allow specific fractions to be collected from a series of runs.

MATERIALS AND METHODS

The chromatography was carried out using a Waters 660 gradient programmer controlling two Waters 6000A pumps. The samples were injected from a Waters Wisp 710B autosampler onto a Waters μBondapak C_{18} column. The column effluent was monitored at 206 nm using a LKB Uvicord S detector fitted with an 8-μl flow cell. The fractions were collected on a LKB Ultrorac II 2070 fraction collector controlled via the external input socket by an Acorn series I microprocessor with a R.A.M. 10 chip fitted at the B2 location (Acorn Computers, Cambridge, Great Britain). The microprocessor was programmed to produce a series of up to 100 output pulses, the interval between each pulse being variable from 1 sec to 2 h. The programme was written in machine code and stored on a domestic cassette tape. An interface was constructed from parts obtained from R.S. Components (Fig. 1). Each microprocessor output pulse stepped the fraction collector via this interface and also caused a negative

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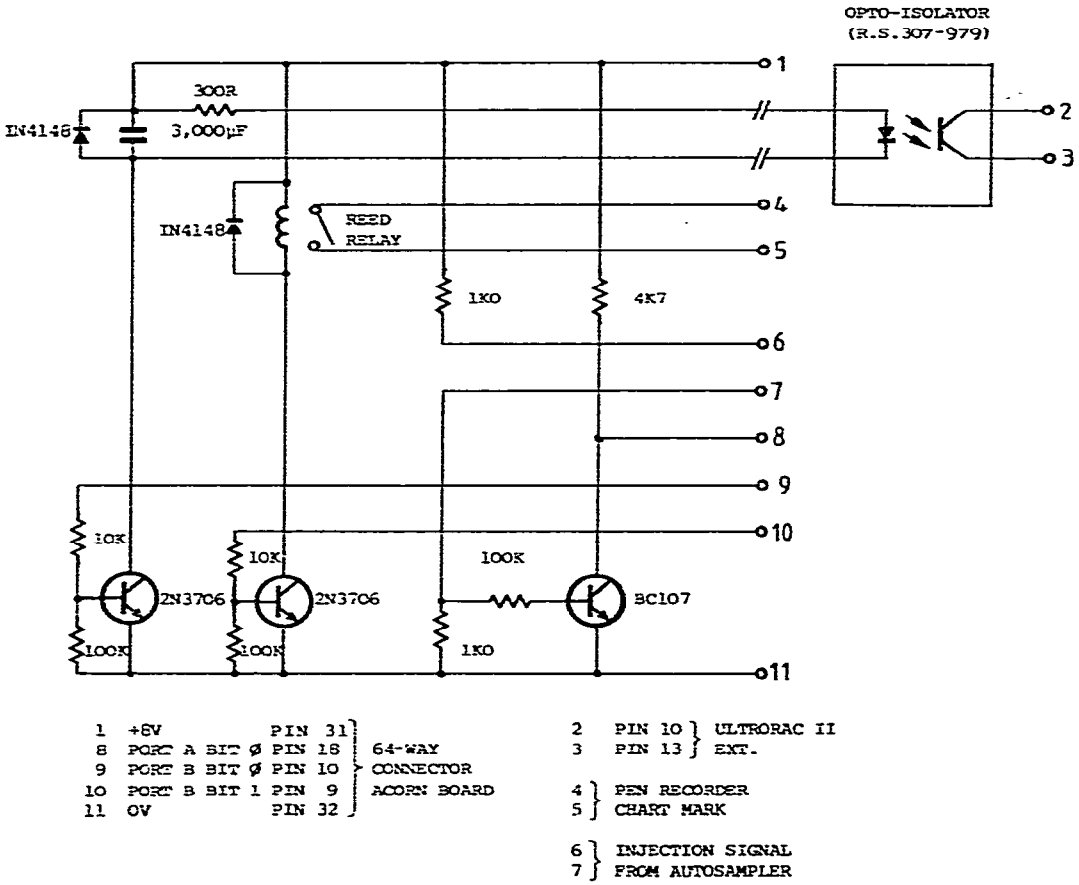

Fig. 1. Circuit diagram of the interface and connections between the autosampler, chart recorder, fraction collector and microprocessor. K = kΩ, R = Ω, 4k7 = 4.7 kΩ, 1KO = 1 kΩ.

(downward) deflection on the chart record. The programme will halt after one run or when linked to an autosampler will recycle for a defined number of runs.

RESULTS

Repeated injections (20 µl) of a standard peptide solution (100 µg/ml) of TRH, LH-RH and substance P were made and from the UV traces the mean elution time for each peptide peak was determined (Table I). After establishing the delay time from detector to collector, the portion of column effluent to be collected was calculated by time using the base width +2 S.D. divided equally about the mid point of the base of the peak (assuming peak symmetry), as the area to be collected for each peak. These times were keyed into the microprocessor. At each step of the fraction collector, a mark was made on the chart. A blank run was then superimposed on the visible standard trace as a means of checking that the collection times fed into the microprocessor correspond with the elution times (Fig. 2).

TABLE I

RETENTION TIMES AND LEVELS OF TRH, LH-RH AND SUBSTANCE P IN THE COLLECTED PEAKS

TRH = thyrotropin releasing hormone; LH-RH = luteinizing hormone releasing hormone.

	Retention time (min)			*Peptide levels (pmol)*		
	x	*S.D.*	*n*	*x*	*S.D.*	*n*
TRH	10.27	0.09	10	1020	26.5	5
LH-RH	14.14	0.10	10	187	9.0	5
Substance P	15.38	0.08	10	177	20.4	5

The reproducibility of the system was assessed by repeated injections of a standard equivalent to levels found in tissue extracts (TRH 1000 pmol, substance P and LH-RH 200 pmol) the collected peaks being assayed using RIA (Table I). Recovery of peptides from the system was calculated using a tritiated peptide ([^{3}H]TRH). 87% of the activity injected on to the column was recovered in one fraction; the mean count of the recovered fraction was 39,577 $\pm$ 957 cpm (n = 10) and the initial activity prior to injection was 45,290 cpm.

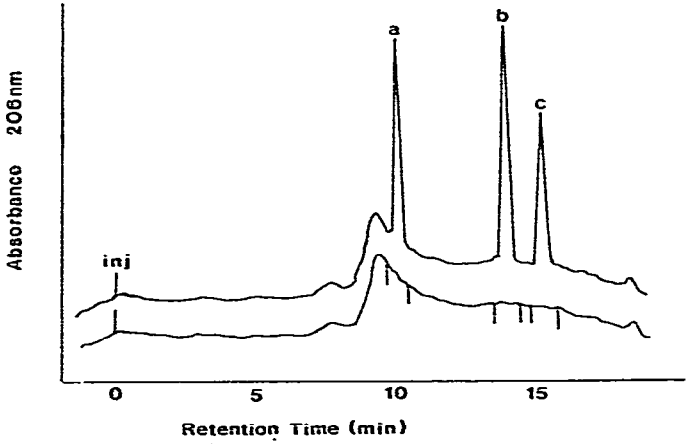

Fig. 2. Separation of TRH, LH-RH and substance P on acetonitrile gradient. Solvent A 0.08% trifluoroacetic acid (TFA); Solvent B 70% acetonitrile with 0.08% TFA; 20 min. Linear gradient from 5 to 70% B. Peaks: a = TRH; b = LH-RH; c = substance P (all 2.0 μg at 0.5 a.u.f.s.).

DISCUSSION

The system described provides a cheap, simple and highly effective means of recovery of compounds eluting in an HPLC system that are either below the limit of on-line detection or are masked by extraneous peaks that are often present in biological preparations. The system is currently in use in our laboratory for the collection and analysis of peptides from tissue extracts and cerebrospinal fluid. The device should be readily adaptable to other HPLC systems, both analytical or preparative, with only minor modification.

The Acorn microprocessor is flexible enough to allow further programming which could include a base line monitor acting as a level sensing device to enable the collection of visible peaks as well as timed fractions. Finally, the controller could be more permanently programmed by placing a pre-programmed (EPROM) chip into the microprocessor which would obviate the need for a cassette storage system.

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