

NOTE NO. 46 - Jan 1 1994

ELECTROCHEMICAL DETECTION, COLUMN SWITCHING, AUTOMATION.

LINEARITY: 1.5 - 1000 ng/mL PLASMA; REPRODUCIBILITY: 6.5%.

In this first report of combining "ISRP column switching... with electrochemical detection," ISRP applicability has been extended and automated, nanogram/mL analysis, accommodated (1).

Analytes: Dopamine agonist (-)-2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin hydrochloride, I; hydroxy-tetralin internal standard, II.

Sample Matrix: Plasma, animal and human

Sample Size: 100 microliters

Columns:

Injection: 5-micron GFF, 10 mm x 3 mm I.D.; ambient temperature.

Analysis: 4-micron C18, 10 cm x 5 mm I.D.; 30° C.

Mobile Phase:

Acetonitrile/buffer*, (X/Y).

Buffer: 0.02 M sodium phosphate, pH 7.5.

	ISRP	C18
X/Y	10/90	65/35
mL/min	1.0	1.5
Residence, min	3.0	1.0

Detection: ESA guard cell and dual-electrode Coulochem

Guard:	+0.8V.	Electrode 2:	+0.6V.
Electrode 1:	+0.37V.		

Discussion: For automated analyses with ISRP columns, column switching (Figure 1) offers two advantages: speed and convenience. Speed: the ISRP column need not be re-equilibrated between samples. Convenience: the concentrator-column selectivity becomes open to choice. Both advantages markedly ease method development.

Finding a marked dependence of agonist peak height on plasma injection concentration and volume, the authors responded pragmatically but effectively: the calibration standards were not spiked aqueous solutions, but spiked plasma. With this approach, they observed a linear response from 1.5 to 1000 ng/mL (Figures 2 and 3). As possible causes for the dependence of peak height on concentration, the authors suggested the shortness of the ISRP column, the low acetonitrile concentration in the injection mobile phase, and the relatively large plasma injection volume. The first and third suggestions may not obtain: already in 1986, Szczerba et al. had reported good success in injecting 100-microliter human serum samples into a GFF guard column (2). The mobile phase compositions differed however, and we would recommend the earlier practice: Szczerba used straight water in injecting, 98% acetonitrile in eluting.

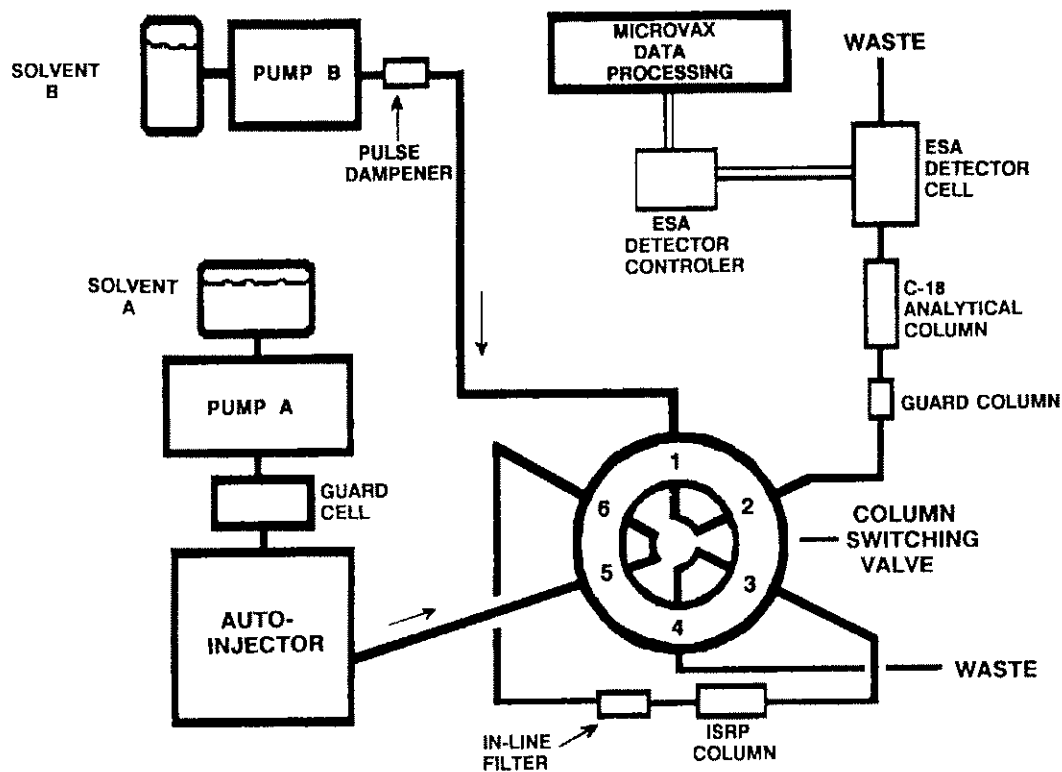


Figure 1. The column-switching valve is shown set for injection; after the valve is indexed, the ISRP-cleaned sample is first charged to the analytical column and then eluted to the detector. (Reproduced from ref. 1 by permission of the Journal of Chromatography.)

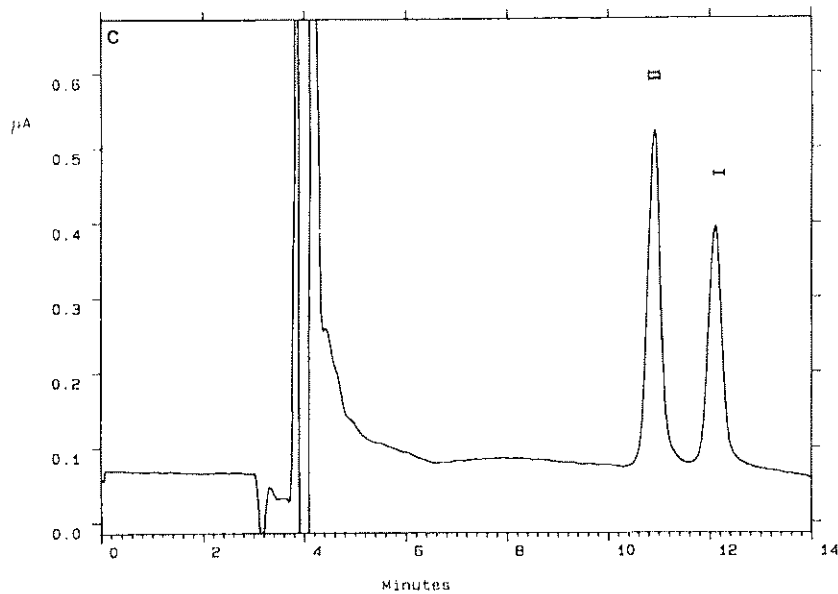


Figure 2. The first peak (II) is that of the internal standard; the second (I), the agonist I at 95 ng/mL concentration. From injection of 100 microliters of plasma from a I-injected monkey. (Reproduced from ref. 1 by permission of the Journal of Chromatography.)

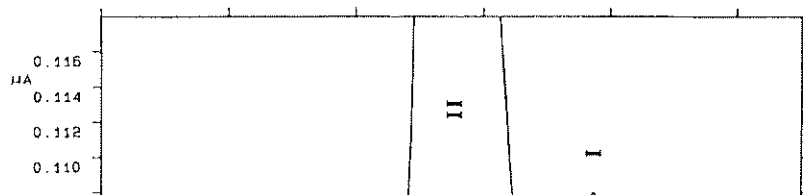


Figure 3. This shows a "chromatogram of spiked human plasma at the detection limit of the method, 1.5 ng/mL

References: (1) DIRECT ANALYSIS OF THE DOPAMINE AGONIST (-)-2-(N-PROPYL-N-2- THIENYLETHYLAMINO)-5-HYDROXYTETRALIN HYDROCHLORIDE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING TWO-DIMENSIONAL COLUMN SWITCHING. Ruckmick, S. C.; Hench, B. D. J. *Chromatogr.* **1991**, *565*, 277-295.

(2) ANALYSIS OF ANTIDEPRESSANTS USING THE CONCENTRATOR COLUMN APPROACH. Szczerba, T. J.; Perry, J. A. Pinkerton Application Note No. 14, August 23, 1986, Regis Chemical Company, Morton Grove, Illinois 60053.

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