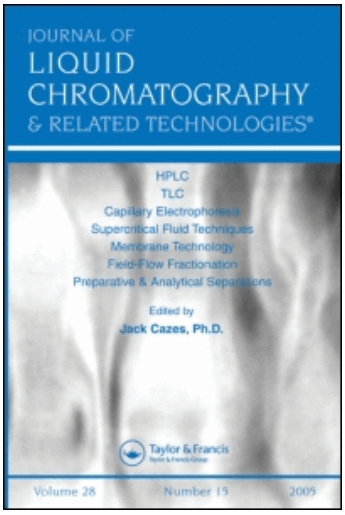

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A Pulse Polarographic Detector for HPLC; Determination of Nitrazepam

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A PULSE POLAROGRAPHIC DETECTOR FOR HPLC; DETERMINATION OF NITRAZEPAM

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ABSTRACT

The advantages of applying a normal pulse technique in polarographic detection are demonstrated in the determination of nitrazepam in artificial solutions and in serum. The low noise level inherent to pulse techniques however, can only be obtained when all additional irregularities and superimposed incidental electrochemical disturbances are eleminated e.g. by an electrochemical scrubber.

INTRODUCTION

Because of the well-known advantages of mercury as electrodematerial, a number of polarographic flow-through cells were developed (1). Fast droprates appeared to be advantageous in view of the signal-to-noise ratio. The technical problem of constructing such a DME detector had been overcome by introducing a conically ground horizontal mercury capillary, resulting in droprates of ca. 25 ms. With direct current amperometry good results were obtained (1,2). However, this fast DME system does not allow the application of sampling techniques such as sampled DC or pulse techniques like normal and differential pulse polarography. All these techniques requi-

re a synchronization of the measurement with the drop lifetime. Synchronization can be achieved by means of drop fall detection or a mechanical device to control the droptime. Each of these techniques has specific disadvantages when used in flow systems. An alternative approach was hence proposed based on a different detector design for droprates from 0.9 to 0.3 s. An instantaneous change in potential induces a variation in surface tension and can cause under certain conditions drop falling. With these conditions established the prinicple is simple to handle and mechanical disturbance of the flow pattern can be avoided (3).

Another problem with these detectors is the background current due to electrochemically active impurities in the eluent stream resulting in a noise in the baseline. As nitrogen purging does not sufficiently suppress this phenomenon, the impurities were removed by means of a flow-through cell with porous silver electrodes. Using such a scrubber the noise due to eluent background effects is significantly decreased and the advantages of the above mentioned pulse and current sampling techniques become apparent to their full extent (4).

To test the system under real conditions, the pulse DME detector with the electrochemical eluent scrubber was employed for the chromatographic determination of nitrazepam in artificial solutions and serum. Nitrazepam is one of the 1,4-benzodiazepines, commonly used as tranquilizer or sedativum.

The results for artificial solutions are compared to those obtained, under similar conditions, with the best of the earlier designed detectors with fast droprate (2).

EXPERIMENTAL

The pulse DME detector.

In this design (see fig. 1) longer droptimes than in the earlier designs (1) were applied. Again a horizontal mercury capillary was used, of which the end was partially ground conical. This shape has excellent positioning and sealing characteristics.

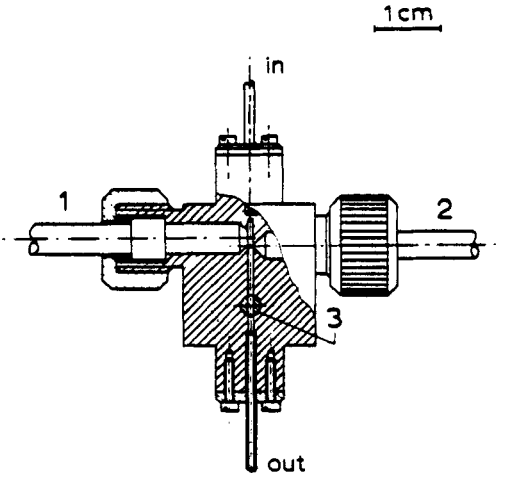

Figure 1 : The pulse DME detector
1: mercury capillary, working electrode;
2: reference electrode;
3: auxilary electrode

The droptimes with a 8 cm long glass capillary are from 0.3 to 0.9 s, dependent on the mercury height and the flowrate. A straight flow channel was chosen in order to avoid tear-off effects on the mercury drop (1). The glass connecting tube of the reference electrode was also conical and, as in earlier designs, attention was paid to a correct positioning of all three electrodes.

For an electronic control of the droptime, a short negative pulse (20 ms, -1 V) is applied in the last stage of the drop lifetime. Once the droptime is controlled by such a "knock" pulse, it is possible to synchronize the normal pulse measurement and the mercury drop (3). Advantages of applying normal pulse compared to direct amperometry, are lower noise and a flow independent signal.

The electrochemical eluent scrubber

This scrubber was designed to be used as flow-through cell in the high pressure part of a chromatographic system. The opti-

mal position for this cell is immediately before the injection valve. Porous electrodes were chosen to ensure a large electrode surface. Silver was chosen as electrode-material because of its favourable behaviour in the reduction of oxygen. The cell is operated in a simple two-electrode system. A detailed description of the design and the performance of the scrubber was given earlier (4).

Apparatus

The chromatographic system consisted of a PE 601 pump (Perkin Elmer, Norwalk, Conn., U.S.A.), an injection valve (25 µl loop) (Valco Instruments Co., Houston, Tex., U.S.A.) and a column (10 cm x 4.6 mm i.d.) packed with LiChrosorb RP-8, 10 µm (Merck, Darmstadt, G.F.R.). The measuring pulse was generated by a Tacussel UAP 4 pulse unit and PRT 30-01 potentiostat (Tacussel, Villeurbanne, France) and the start of this pulse triggered via a homemade interface a PAR 175 programmer (Princeton Applied Research Co., Princeton, N.J., U.S.A.) for the additional "Hg-Drop" pulse. The electrical currents were measured with the Tacussel PRT 30-01/UAP 4 and recorded at a HP 7046 dual pen XY-recorder (Hewlett Packard, San Diego, Cal., U.S.A.). The potential pulse program and the electrical currents were controlled on a Tektronix 5103 N oscilloscope (Tektronix, Beverton, Ore., U.S.A.). The mercury capillary was delivered by Metrohm (1091/1, Metrohm, Herisau, Switzerland) and machined to the desired shape in our workshop. The DME detector compartment was made of Plexiglass, the auxilary electrode of platinum. The applied potential was always measured vs. a Ag/AgCl/1 M LiCl, methanol-water (50/50) reference electrode.

The eluent scrubber was made in our workshop. The voltage on the silver electrodes was applied by a Wenking 68 FR 05 Potentiostat (Gerhard Bank Elektronik, Göttingen, G.F.R.) and controlled on a digital voltmeter.

Chemicals

In every measurement water-methanol (50/50, v/v) was used as eluent, which contained 0.1 M KNO_3 and 10^{-3} M HNO_3. The stock

solution of this eluent was continuously de-aerated by purging with nitrogen (A28). Lithiumchloride, methanol, potassium nitrate, nitric acid, nitrobenzene, perchloric acid and sodium carbonate were Baker "Analyzed" chemicals (J.T. Baker, Phillipsburg, N.J., U.S.A.). Nitrazepam was supplied by Nogefa (Haarlem, the Netherlands). All chemicals were used without further purification.

RESULTS AND DISCUSSION

Determination of nitrazepam

Nitrazepam (1,3 dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2 one, mol. wt. 281.26) possesses two reducible functional groups. The first one is the nitrogroup ($E_{1/2}$ = -325 mV, in the eluent used, flowrate 1 ml/min) and the second the azomethine group ($E_{1/2}$ = -925 mV, same conditions). The determination of nitrazepam was based on the reduction of the nitrogroup since a low applied potential is favourable for selectivity and noise.

For the comparison of the performance of the pulse DME detector and the fast DME detector (2) nitrazepam in artificial solutions was chromatographed with and without the electrochemical eluent scrubber under otherwise identical conditions.

Artificial solutions

The results for both detectors are presented in table I and figures 2 and 3. The oxygen present in the sample was easily separated from nitrazepam. The detection limits were determined for a signal-to-noise ratio of 3 with the noise measured as peak-to-peak variation in the baseline. The background current is the current measured when only the eluent is pumped through the system.

As can be seen in Figure 2 and Table I the effect of the scrubber on the noise and the detection limit is small in case of the fast DME detector, since only the background current is decreased. Apparently the fast droprate brings already an optimization of the signal-to-noise ratio.

Using the electrochemical eluent scrubber with the pulse DME detector results in a significant decrease of the background

TABLE I

Comparison of the fast DME and pulse DME detector

Detector	Without scrubber	With scrubber (-1.4 V)
Background current (nA)		
Fast DME	560	80
Pulse DME	950	75
Noise (nA)		
Fast DME	0.45	0.40
Pulse DME	1.30	0.15
Detection limit (ng/inj.)		
Fast DME	4	4
Pulse DME	25	1

N = 1400; K' (nitrazepam) = 4.5. (Conditions: see Figures 2 and 3)

current and noise (see Table I and Figure 3). The stability of the baseline is also greatly improved. With the large mercury drops used in this detector design the noise is predominantly determined by the electrochemically active impurities in the eluent stream. Hence lowering the concentration of these impurities decreases of the background current and the noise. The advantages of applying a pulse technique becomes obvious, looking at the detection limits in Table I.

The pulse DME detector, used with the electrochemical eluent scrubber, exhibits an excellent linear range for nitrazepam. A regression coefficient of 0.9996 for a range from 7.0 to 70 ng per injection (25 µl) and of 0.9998 for a range from 70 to 700 ng per injection was observed. The repeatability for this deter-

mination was 1.8% (relative standard deviation) (N=15), which is 0.3% better than that of a fast DME detector (2.1% r.s.d.).

Serum

Many papers have been published on the determination of benzodiazepines in biological fluids with various techniques such as GC (reviews of Hailey (6) and Clifford and Smyth (7), voltammetry (review of Smyth and Smyth (5)) and HPLC with UV detection (8,9). The advantage of the determination via reversed phase chromatography is the relatively simple sample preparation and the adaptability of the method for electrochemical detection. It was found, that the extraction method applying Extrelut (Merck) (8) was not suitable for electrochemical detection. In the nanogram range using small (1 ml) serum samples we could not completely elute nitrazepam from the extraction column. The following procedure proved to be successful: add 50 µl concentrated perchloric acid to 1 ml serum and shake for 1 min. After centrifugation (5 min, 3500 cpm) the supernatant layer is taken and neutralized with 50 µl 30% sodiumcarbonate solution. The neutralized solution was injected directly (see Figure 4). The recovery was 67 ± 3% when corrected for the dilution. The detection limit was the same as in artificial solutions (1 ng per injection) taking into account the dilution and the recovery of 67%.

CONCLUSIONS

With the design discussed here the difficulties of applying pulse techniques in polarographic detection for HPLC have been overcome. The advantages of a pulse DME detector become apparent if this detector is used in combination with an electrochemical eluent scrubber. Only after removal of electrochemically active impurities in the eluent stream is it possible to take advantage of the low noise level inherent to pulse techniques. Low detection limits are attainable then. The selectivity of polarographic detection permits quantitation of nitrazepam in serum with a minimum of sample preparation.

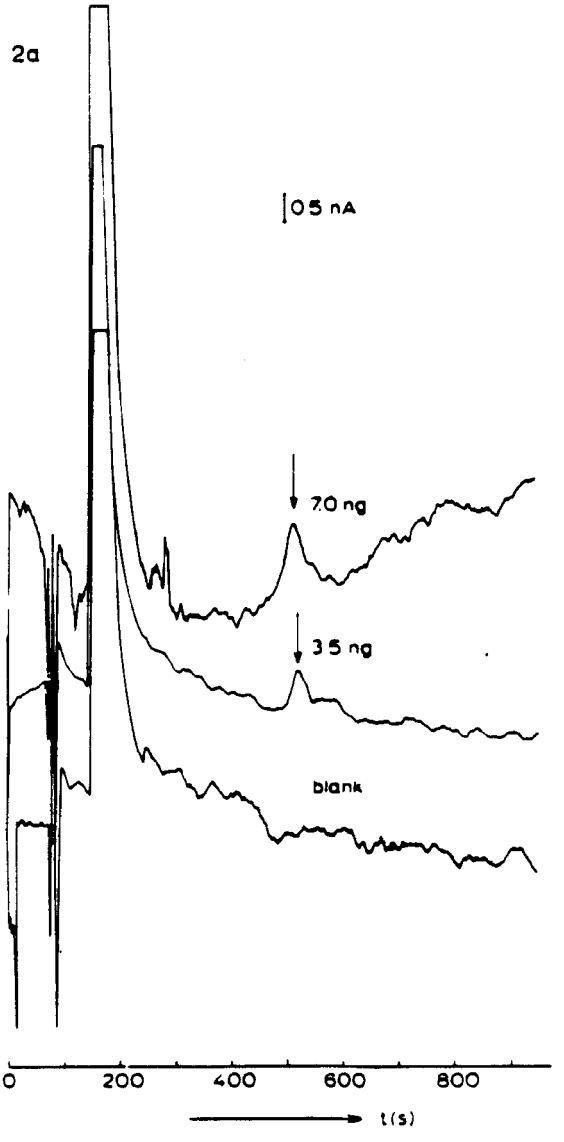

Figure 2a : Performance of the fast DME detector on the determination of nitrazepam, without the electrochemical scrubber. Conditions: flowrate 1 ml/min; eluent: 50/50 methanol--water (v/v), 0.1 M KNO_3, 10^{-3} M HNO_3; droptime: 15 ms, mercury height: 76 cm, time constant: 0.5 s, E = -600 mV

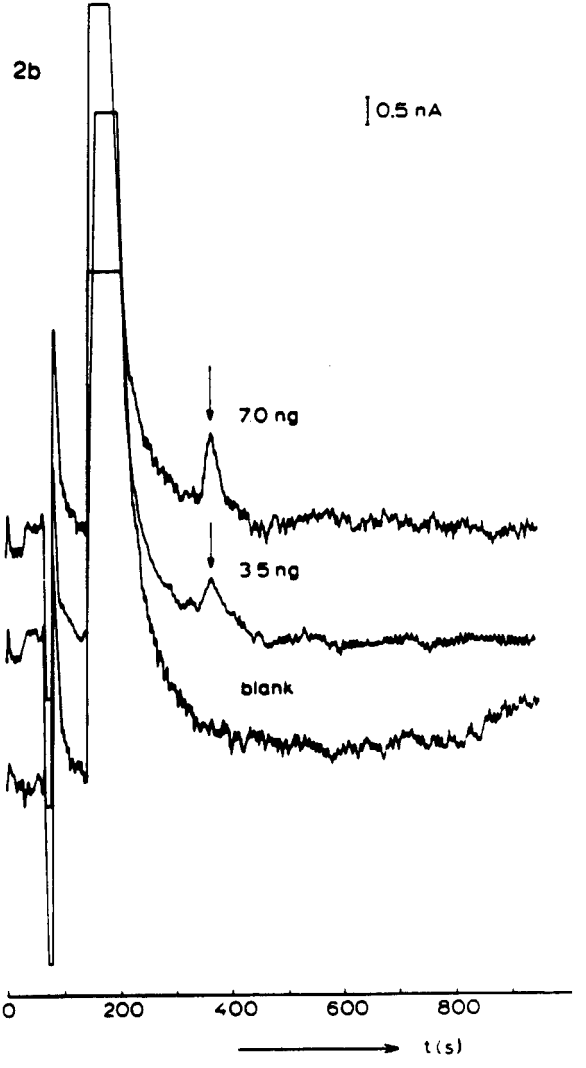

Figure 2b : Performance of the fast DME detector on the determination of nitrazepam, with the electrochemical scrubber. Conditions: scrubber potential: -1.5 V, further conditions see Figure 2a.

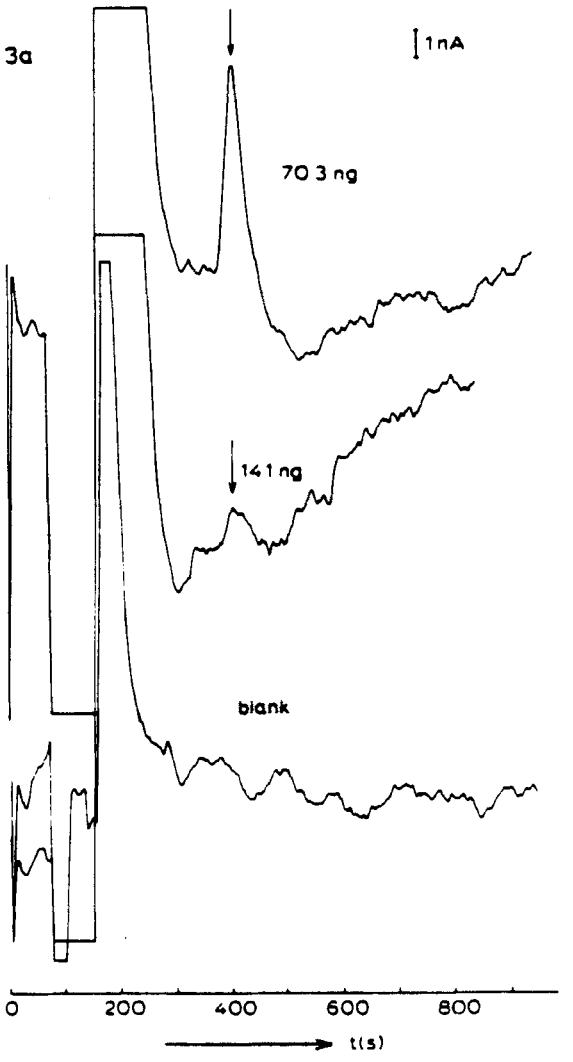

Figure 3a : Performance of the pulse DME detector on the determination of nitrazepam, without the electrochemical scrubber. Conditions: pulse: -100 mV to -400 mV, free droptime 0.35 s, cycletime: 0.32 s, delay: 0.04 s, pulse: 0.25 s, sample window: 20-60%, time constant 0.1 s; further conditions see Figure 2.

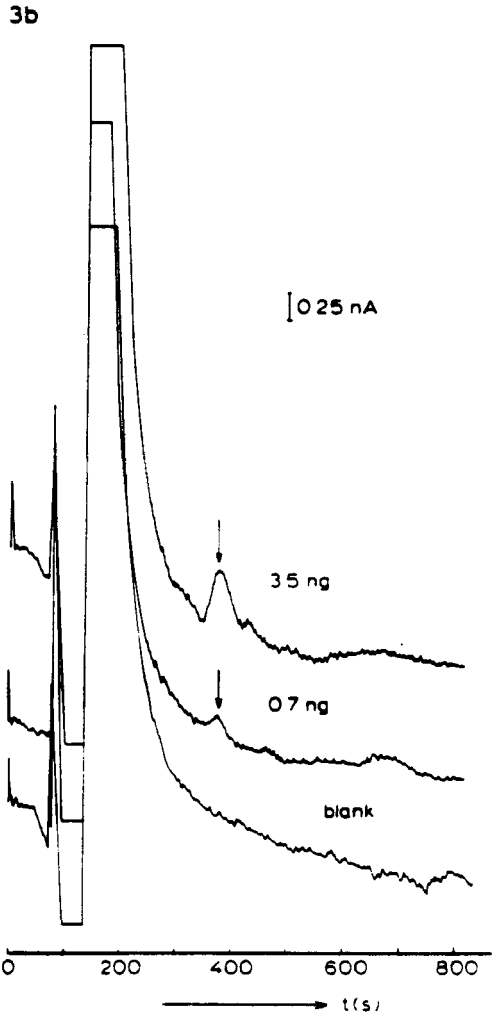

Figure 3b : Performance of the pulse DME detector on the determination of nitrazepam with the electrochemical scrubber. Conditions: scrubber potential: -1.5 V; further conditions see Figure 3a.

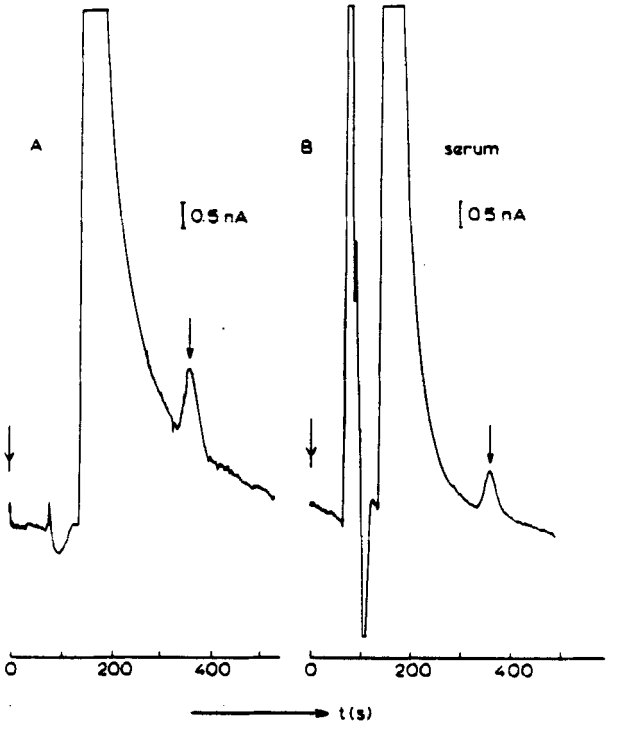

Figure 4 : Comparison of the determination of nitrazepam in a) artificial solution and b) serum (pulse DME detector with electrochemical scrubber) both spiked (serum before sample treatment) with 7 ng/25 µl. (Indicated peak).
Conditions: see Figure 3.

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