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USE OF CATION EXCHANGERS FOR THE ON-LINE PRECONCENTRATION OF POLAR ANILINES IN LIQUID CHROMATOGRAPHY

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SUMMARY

On-line preconcentration (for use in liquid chromatography) of polar solutes such as substituted anilines cannot conveniently be carried out on conventional alkyl-modified silicas, or other, resin-based, hydrophobic materials because of insufficient retention. Small precolumns packed with a resin-based strongly acidic cation exchanger were successfully used for the preconcentration of nine polar anilines, with subsequent determination by liquid chromatography with UV absorbance or electrochemical detection.

Because of the low capacity of the ion exchanger and the presence of relatively high concentrations of ionic compounds in the surface water samples tested, it was necessary to introduce a clean-up step consisting of oxalate precipitation of calcium(II). With electrochemical detection, detection limits for the anilines are *ca.* 0.2–5.0 ng. In the analysis of river water samples, this corresponds to a detection limit of 0.02–0.5 ppb (10^9).

INTRODUCTION

Sample pretreatment based on liquid–solid sorption techniques has been shown[1] to be very useful for preconcentration of environmental samples in liquid chromatography (LC). In our laboratories, C_{18}-modified silica[2], styrene–divinylbenzene copolymers[3] and carbon-based[4] sorbents have been used for on-line trace enrichment of many non-polar and moderately polar solutes from aqueous samples, utilizing precolumns with geometrical volumes of 30–80 μl. Further work has shown, however, that such a set-up cannot be used for the efficient preconcentration of highly polar compounds from sufficiently large sample volumes. Such volumes, often arbitrarily set at *ca.* 10 ml, guarantee an enrichment factor of *ca.* 100 compared with a conventional 100-μl loop injection.

In the literature, precolumns packed with cation exchangers have been recommended for on-line trace enrichment of metal ions from nuclear reactor coolants and natural waters[5], and of amino acids and polyamines from blood and urine samples by means of an automated amino acid analyzer[6]. Sturgeon *et al.*[7] used columns containing silica-immobilized 8-hydroxyquinoline for the preconcentration of trace elements from sea water.

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Lores and co-workers[8,9] reported various LC procedures with electrochemical detection (ED) for the determination of halogenated anilines and related compounds with detection limits in urine of below 5 ppb*. A recent paper[10] describes the LC separation of phenylenediamines on a 15-cm column containing 3-μm C_{18}-modified silica, but no attempts to analyse real samples are presented. Kaczinsky *et al.*[11] used off-line preconcentration on a sulphonated XAD-4 resin, subsequent desorption with a mixture of methanol and ammonia, concentration via evaporation and final analysis by means of gas chromatography (GC). Over 50 organic bases were analysed with good recovery at the 1-ppm and 50-ppb levels, but no detection limits are given. Riggin *et al.*[12] evaluated the determination of aniline and its derivatives in waste water by means of GC and LC. However, aminophenols and phenylenediamines were not included in their study. The authors prefer GC with termionic nitrogen–phosphorus detection to LC with UV detection because of the distinctly higher sensitivity of the GC procedure (1–12 ppb). Finally, Riggin and Howard[13] developed a method for the determination of phenylenediamines in aqueous samples. Here, isolation on a strongly acidic cation exchanger preceded ion-pair LC with ED. However, no real preconcentration occurs in this procedure, and the limits of detection are still 10–60 ppb.

Obviously, there is still a need for an easily automatable LC method for the determination of polar anilines in real aqueous samples, *i.e.* in the presence of cationic and/or non-polar contaminants. In this paper, we describe such a procedure, which combines on-line preconcentration on a short precolumn, packed with a suitable cation exchanger, and separation on a C_{18}-bonded phase with ED.

EXPERIMENTAL

Apparatus

A Kontron (Zürich, Switzerland) LC system, consisting of two Model 410 pumps, a Model 200 programmer and MCS 670 Tracer switching unit, was used. In experiments aimed at obtaining maximum sensitivity, a laboratory-built syringe pump was used. A variable-wavelength LC 55 spectrophotometer (Perkin-Elmer, Norwalk, CT, U.S.A.) served as the UV detector. The electrochemical detector consisted of a Metrohm (Herisau, Switzerland) 1096/2 cell, equipped with a glassy carbon working electrode, a Ag/AgCl/1 *M* LiCl (in 50% methanol) reference electrode, and a copper or platinum auxiliary electrode, and a laboratory-built potentiostat/amplifier. Chromatograms were recorded on a W + W 900 (Kontron) recorder.

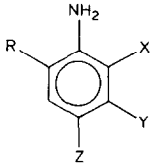
Stationary phases and columns

Preconcentration was accomplished on laboratory-packed[14] 2 × 4.6, 4 × 4.6 and 5 × 3.0 mm I.D. stainless-steel precolumns, which are also commercially available from Chrompack (Middelburg, the Netherlands). The precolumns were packed by using a microspatula, with the spherical 10-μm styrene–divinylbenzene copolymer PRP_1 (Hamilton, Reno, NV, U.S.A.) or a sulphonic acid-type silica-based (Merck SCX, 10 μm; Merck, Darmstadt, F.R.G.) or resin-based (Aminex A-7, 9 μm, Bio-Rad, Richmond, VA, U.S.A.) cation exchanger.

* Throughout this article, the American billion (10^9) is meant.

TABLE I

SUBSTITUTED ANILINES USED AS TEST COMPOUNDS



Compound	*X*	*Y*	*Z*	*R*	*Supplier*
Aniline	H	H	H	H	Aldrich
o-Phenylenediamine	NH_2	H	H	H	Fluka
m-Phenylenediamine	H	NH_2	H	H	Fluka
4-Methyl-*m*-phenylenediamine	H	NH_2	CH_3	H	Fluka
o-Toluidine	CH_3	H	H	H	Fluka
o-Anisidine	OCH_3	H	H	H	Fluka
p-Anisidine	H	H	OCH_3	H	Fluka
p-Chloroaniline	H	H	Cl	H	Fluka
p-Aminophenol	H	H	OH	H	Merck
3-Amino-4-ethoxyacetanilide	H	$NHCOCH_3$	H	OC_2H_5	Unknown

The analytical column was a 25 cm × 4.6 mm I.D. stainless-steel, or a 20 cm × 3.0 mm I.D. glass column, prepacked with 8-μm CP-Spher C18 (Chrompack).

Chemicals

Analytical-grade methanol, phosphoric acid, perchloric acid, potassium nitrate, potassium monohydrogen phosphate, citric acid and oxalic acid were obtained from J. T. Baker (Deventer, the Netherlands). EDTA was obtained from Sigma (St. Louis, MO, U.S.A.) and potassium citrate from ACF (Maarsen, the Netherlands). Demineralized water was purified in a Milli-Q (Millipore, Bedford, MD, U.S.A.) filtration system to obtain LC-grade water for use in mobile phases and standard solutions. Eluents were degassed in an ultrasonic bath under vacuum.

The polar anilines used as test compounds, and their suppliers, are shown in Table I. Stock solutions were prepared by weighing the anilines and dissolving them in methanol. These solutions were diluted with LC-grade water adjusted to pH 3, to obtain standard solutions at the ppb level.

RESULTS AND DISCUSSION

Preconcentration

Breakthrough curves for PRP_1, Merck SCX and Aminex A7 columns were recorded according to the procedure outlined in ref. 2, with 250-ppb standard solutions of pH 3 at a flow-rate of 5 ml min^{-1}. The results are reported in Table II. Obviously, polar anilines cannot be concentrated from 10-ml sample volumes on the PRP_1 material. The only compound with appreciable retention is *p*-chloroaniline. Preconcentration of polar anilines on PRP_1 is more efficient under neutral conditions, as described elsewhere[15], but the breakthrough volumes are still rather low (0.3–24 ml). At pH 3, PRP_1 can be used as an effective clean-up filter, trapping non-polar

TABLE II

BREAKTHROUGH VOLUMES OF POLAR ANILINES ON SHORT PRECOLUMNS, PACKED WITH VARIOUS SORBENTS

LC-water samples, containing 250 ppb of test solute; pH adjusted to 3.0 with perchloric acid; sampling rate, 5 ml min^{-1}.

Compound	*Breakthrough volume (ml) on:*		
	PRP_1, 10 μm 2 × 4.6 mm I.D.	*Merck SCX, 10 μm* 4 × 4.6 mm I.D.*	*Aminex A-7, 9 μm* 4 × 4.6 mm I.D.*
Aniline	0	17	> 100
p-Phenylenediamine	0	> 100	> 100
m-Phenylenediamine	0	> 100	> 100
4-Methyl-*m*-phenylenediamine	1	> 100	> 100
o-Toluidine	1	65	> 100
o-Anisidine	0	34	> 100
p-Anisidine	0	34	> 100
p-Chloroaniline	6–10	–**	> 100
p-Aminophenol	0	9	> 100
3-Amino-4-ethoxyacetanilide	2	77	> 100

* Maximum values (see text).
** Not determined.

neutral contaminants without affecting the trace enrichment of the anilines on other column materials.

The silica-based Merck and, even more so, the resin-based Aminex cation-exchange columns, preconcentrate the protonated polar anilines well, with breakthrough volumes of more than 30 ml in all but two cases (Table II). Unfortunately, these breakthrough volumes have to be considered as maximum values. The depen-

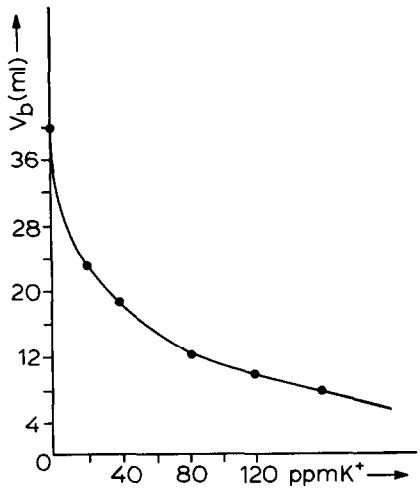


Fig. 1. Dependence of the breakthrough volume (V_b) of 3-amino-4-acetanilide on the ionic strength of the sample solution at pH 2.6. Solute concentration, 500 ppb; flow-rate, 2 ml min^{-1}; precolumn, 4 × 4.6 mm I.D., packed with Merck SCX.

dence of breakthrough volumes on the ionic strength of the sample solution was studied by recording breakthrough curves for 500-ppb solutions of 3-amino-4-ethoxyacetanilide at pH 2.6 to which increasing amounts of potassium nitrate had been added. The experiments were performed with the silica-based cation exchanger at a flow-rate of 2 ml min^{-1}. The breakthrough volume decreases dramatically with increasing ionic strength (Fig. 1). If a breakthrough volume of 10 ml is taken to be the lowest permissible value, then the highest allowable concentration of potassium is only 120 ppm. Bivalent ions, such as calcium(II), reduce the breakthrough volume to 10 ml at a concentration of 8 ppm. Passing several millilitres of a dilute solution of calcium(II) through a precolumn made on-line regeneration almost impossible: 20 ml of 10^{-3} *M* perchloric acid did not suffice to restore the original sorption quality of the cation exchanger. Finally, it should be noted that the breakthrough volume of 3-amino-4-ethoxyacetanilide in the absence of potassium nitrate decreases with decreasing pH. At pH 3.0 the breakthrough volume was 77 ml (Table II) and at pH 2.6 it was 40 ml (Fig. 1).

The low capacity of the silica-based cation exchanger prevents its use in small (4 × 4.6 mm I.D.) precolumns for the preconcentration of the anilines from real samples. The resin-based exchanger, which has a much higher capacity, was therefore used in all further work. A simple additional clean-up step was also introduced to reduce the negative influence of ionic sample constituents on breakthrough. It consists of precipitation of calcium(II) with oxalic acid and complexation of traces of iron(III) with EDTA. The procedure was developed with Amsterdam tapwater, which contains *ca* 100 ppm of calcium(II). Addition (per 100 ml of tap water) of 1.5 ml of a solution containing 2.4 mg of oxalic acid per ml and 1 ml of a solution containing 18 mg of EDTA per ml gave adequate results. To quote an example, after addition of the reagents to a spiked tap water sample, subsequent filtration through a 0.8-μm membrane filter, and adjustment of the solution to pH 3.0, the breakthrough of the spike, 4-methyl-*m*-phenylenediamine, on a 4 × 4.6 mm I.D. precolumn, packed with Aminex A-7, was more than 80 ml, which was the maximum volume tested. In another experiment, three consecutive 10-ml preconcentrations were carried out using the same spiked tap water; the precolumn was regenerated on-line with 20 ml of 0.02 *M* perchloric acid. Recoveries of over 90% were observed each time, indicating that the column had been successfully regenerated. The simple clean-up step does not impair the usefulness of the proposed method, since environmental samples are generally filtered anyway.

Desorption

For the desorption of aniline from the cation exchanger, 0.07 *M* aqueous potassium monohydrogen phosphate (adjusted to pH 7)–methanol (7:3) was used. For efficient desorption a fairly high potassium concentration of 0.1 *M* was found to be necessary. With a 0.1 *M* sodium (instead of a potassium) phosphate buffer the peaks were relatively broad and they tailed severely.

To prevent band broadening, the retention in the precolumn should be equal to or less than that in the analytical column. Unfortunately, the resin-based cation exchanger displays a stronger reversed-phase interaction with the anilines than does the C_{18}-bonded phase in the separation column. The resin-based exchanger will therefore cause more band broadening than the silica-based cation exchanger. How-

ever, aspects such as precolumn capacity and ease of column regeneration (see above) still make Aminex A-7 preferable to the silica-based exchanger. Backflush rather than forward flush desorption should be used because backflush seems to cause less broadening. The final gain in sensitivity was determined by comparing the peak heights obtained with 100-μl loop injections of a 1000-ppb solution of 4-methyl-*m*-phenylenediamine with those of the same solute enriched from 10 ml of a 10-ppb solution. Because of the additional band broadening (see above), the sensitivity was increased by preconcentration only 50- instead of 100-fold.

Final procedure

The automated analysis of the polar anilines was carried out with the experimental set-up shown in Fig. 2. The following procedure was adopted. (1) Precipitation and complexation of interferences by oxalate and EDTA, respectively. (2) Filtration and adjustment to pH 3.0. (3) Simultaneous clean-up and concentration on PRP_1 and Aminex A-7, respectively. (4) Flushing of the cation exchanger with water. (5) Backflush desorption from the cation exchanger to the C_{18} analytical column. (6) Flushing of PRP_1 with methanol. (7) Regeneration of cation exchanger with 0.02 *M* perchloric acid. (8) Regeneration of PRP_1 and flushing of cation exchanger with 10^{-3} *M* perchloric acid. (Note that steps 3–8 are fully automated; the time-based column switching programme is given in the Appendix.)

The PRP_1 precolumn upstream of the cation-exchange precolumn was included to act as a filter for non-polar and moderately polar compounds. In the absence of the PRP_1 precolumn, such compounds will be trapped by the resin matrix of the

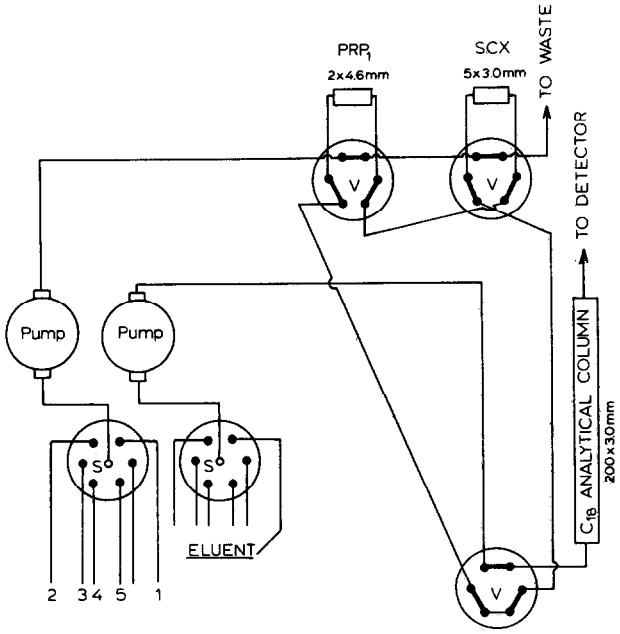


Fig. 2. Experimental set-up for the on-line preconcentration of water samples according to the final procedure described in the text. S = Low-pressure selector valve; V = high-pressure switching valve; 1 = sample; 2 = water; 3 = methanol; 4 = 0.02 *M* $HClO_4$; 5 = 0.001 *M* $HClO_4$.

cation exchanger and reduce the available ion-exchange capacity. As has been explained before, the protonated anilines are not significantly retarded on PRP_1 under the selected experimental conditions (Table II).

When large series of filtered tap or river water samples were handled, a gradual increase of the pressure over the analytical column was observed. This may have been caused by the presence of cationic sample constituents which precipitate as their phosphate salts. We therefore replaced the potassium phosphate by 0.07 *M* potassium citrate, adjusted to pH 6, which was used without any problem in all further work.

In the final procedure, a highly efficient 20 cm × 3.0 mm I.D. C_{18}-type separation column was used (at a flow-rate of 0.4 ml min^{-1}) instead of the earlier 25 cm × 4.6 mm I.D. column. This led to a two-fold increase in sensitivity, and to reduction of more than 50% in solvent consumption. It was found to be essential to match the inner diameter of the cation-exchange precolumn to the separation column (3 mm); otherwise very bad peak shapes will be obtained. Presumably, the highly different retention characteristics of the stationary phases in the precolumn and the analytical column play an important role here.

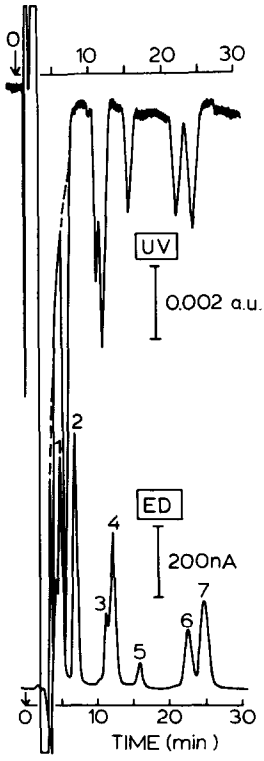


Fig. 3. LC of a standard solution containing 10 ppb of each of the compounds in Table I, except for the late-eluted *p*-chloroaniline; 10 ml of sample concentrated on a 5 × 3.0 mm I.D. Aminex A-7 precolumn. Analytical column: 20 cm × 3.0 mm I.D. CP-Spher C_{18}. Eluent: 0.07 *M* potassium citrate (pH 6)–methanol (7:3) at 0.4 ml min^{-1}. Detection by UV absorbance at 235 nm (attenuation, 0.02 a.u.f.s.) and, in series, electrochemically at +0.9 V (attenuation, 2 μA f.s.). 1 = *p*-Aminophenol + *m*-phenylenediamine; 2 = *o*-phenylenediamine + 4-methyl-*m*-phenylene-diamine; 3 = *p*-anisidine; 4 = aniline; 5 = 3-amino-4-ethoxyacetanilide; 6 = *o*-anisidine; 7 = *o*-toluidine.

Detection

Fig. 3 shows chromatograms of a standard solution containing 10 ppb of all test compounds except for the late-eluted *p*-chloroaniline. Two detectors in series, a UV-absorbance detector operated at 235 nm and an electrochemical detector operated at +0.9 V were used. The latter detector has higher sensitivity for the anilines (*cf.*, ref. 13) and better selectivity for the early-eluted test solutes. At the optimum oxidation potential, of +0.9 V, determined in preliminary experiments, the background current is low and the baseline therefore highly stable, which improves the signal-to-noise ratio of the anilines.

Repeatability ranged between ±1 and ±9% relative standard deviation (n = 4) at the 10-ppb level and was the same for loop injections and preconcentration experiments; this shows the excellent performance of the precolumn system. Detection limits with the electrochemical detector were between 0.2 and 5.0 ng for all test compounds. Method detection limits are summarized in Table III. The calibration curve for aniline was linear (r = 0.9989; six data points) over the whole range tested (0.5–50 ppb).

Application

Fig. 4 shows chromatograms of a blank Amstel (Amsterdam, The Netherlands) river water sample and the same sample spiked to 0.5 ppb with all but one of the test compounds. The early-eluted compounds are not easily detected because of the strong background in this region. The non-spiked river water contains *ca.* 0.5 ppb of aniline, *p*-anisidine and *o*-toluidine. Rather surprisingly, the phenylenediamines are hardly visible in the spiked sample. This is not due to the oxalate precipitation step or to coprecipitation, as was demonstrated in analyses of tap water samples with which no such complications occurred.

TABLE III

DETECTION LIMITS FOR ELECTROCHEMICAL DETECTION OF POLAR ANILINES IN THE PRECONCENTRATION/SEPARATION PROCEDURE

For conditions, see Fig. 4 and text.

Compound	*ED limit (ppb)**	*UV detection limit (ppb)**
Aniline	0.03	1.0
o-Phenylenediamine 4-Methyl-*m*-phenylenediamine	0.02	0.5
m-Phenylenediamine *p*-Aminophenol	0.04	—**
o-Toluidine	0.07	2.0
o-Anisidine	0.10	2.5
p-Anisidine	0.06	1.5
3-Amino-4-ethoxyacetanilide	0.50	2.5

* Signal-to-noise ratio, 3:1.

** Not determined.

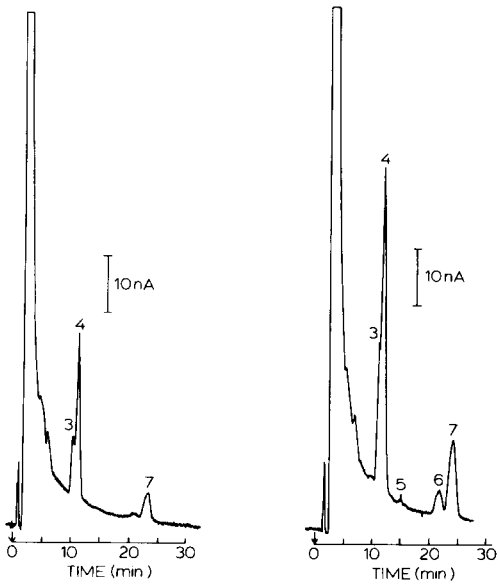


Fig. 4. Left: LC of a blank Amstel river water sample. Right: LC of an Amstel river water sample spiked with 0.5 ppb of each of the compounds in Table I except *p*-chloroaniline. Eluent pump, non-pulsating laboratory-built syringe pump. ED at +0.9 V (attenuation, 100 nA f.s.). Other conditions and identification as in Fig. 3.

CONCLUSIONS

Polar anilines can be preconcentrated on-line on a strongly acidic cation exchanger, provided a rapid clean-up step (oxalate precipitation and PRP_1 precolumn) is used to remove most of the interferences. The method can easily be automated and has sub-ppb detection limits for real samples when LC with ED is used.

Now that the preconcentration and on-line determination of these analytically problematic aromatic anilines have successfully been performed, an interesting next development will be to combine this knowledge with our know-how about on-line preconcentration of relatively non-polar compounds on PRP_1 and C_{18}-bonded phases. This should enable the development of an automated system for the on-line determination of a wide variety of cationic, neutral and anionic pollutants. The set-up of such a system and its application to the analysis of industrial waste water is presently being studied.

ACKNOWLEDGEMENT

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APPENDIX

Automated procedure. Equipment: Kontron Model 200 programmer, Kon-

tron MCS 670 Tracer switching unit; analytical column, 20 cm × 3.0 mm I.D. CP-Spher C_{18}. Eluent, 0.07 *M* potassium citrate (pH 6.0)–methanol (7:3); flow-rate, 0.4 ml min^{-1}. Precolumn: 2 × 4.6 mm I.D. (PRP_1) and 5 × 3.0 mm I.D. (Aminex A-7); flow-rate as indicated below.

Time (min)	*Flow-rate (ml min^{-1})*	*Call file No.*	*Event*
0.0	8.0	88	Reset; flush capillaries with sample
0.7	2.5		
0.8	2.5	83	Sample over PRP_1 (4 ml)
2.4	1.7		
2.5	1.7	84	Sample over PRP_1 and Aminex A-7 (10 ml preconcentration)
8.5	0.0	94	
8.6	0.0	93	
8.7	0.0	86	Switch to water
8.8	8.0		Flush capillaries with water
9.5	1.0		
9.6	1.0	84	Flush Aminex A-7 with 1 ml of water
10.6	0.0		
10.7	0.0	83	
10.8	0.0	85	Flush capillaries with 0.4 ml of eluent
11.8	0.0	94	Desorb Aminex A-7 with 2 ml of eluent
16.8	0.0	95	
16.9	0.0	93	
17.0	0.0	86	Switch to methanol
17.1	8.0		Flush capillaries with methanol
17.8	2.5		
17.9	2.5	83	Flush PRP_1 with 1.5 ml of methanol
18.5	0.0	93	
18.6	0.0	86	Switch to 0.02 *M* perchloric acid
18.7	8.0		Flush capillaries with perchloric acid
19.4	1.7		
19.5	1.7	84	Regenerate Aminex A-7 with 20 ml of perchloric acid
31.5	0.0	94	
31.6	0.0	86	Switch to 10^{-3} *M* perchloric acid
31.7	8.0		Flush capillaries with perchloric acid
32.4	2.5		
32.5	2.5	83	Regenerate PRP_1 with 5 ml of perchloric acid
34.5	1.7		
34.6	1.7	84	Regenerate PRP_1 and flush Aminex A-7 (5 ml)
37.6	0.0		
37.7		End	

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