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Pulsed electrochemical detection of alkanolamines separated by multimodal high-performance liquid chromatography

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Abstract

Pulsed electrochemical detection (PED) is applied to alkanolamines separated by high-performance liquid chromatography (HPLC). A multimodal HPLC column with both cation-exchange and reversed-phase retention modes is used with an acidic mobile phase to assure alkanolamines are in their cationic form. Baseline resolution of alkanolamines, including positional isomers, is possible. Detector response for a representative alkanolamine, tris(hydroxymethyl)aminomethane (Tris), is shown to be linear over a concentration range of more than three decades. The limit of detection for Tris is 20 nM (500 fmol in a 25-μl injection) and the standard deviation of the PED response for 10 μM Tris is better than 0.4%. HPLC–PED is demonstrated to permit the sensitive and precise determination of alkanolamines in a biological sample (blood) and a commercial formulation (shaving gel) with minimal sample preparation.

1. Introduction

Alkanolamines are used extensively by chemical and pharmaceutical industries as lubricants, corrosion inhibitors, emulsifying agents, and as ingredients of various pharmaceutical preparations. Furthermore, they are often utilized for metal surface finishing, gas purification, and as additives and dyes in cleaning solutions [1,2]. Because alkanolamines are used in many ways, and since they have been identified as pollutants in certain waste water effluents [3], there is a strong need to quantify alkanolamines with high sensitivity and accuracy, and without extensive sample manipulation.

Methods used to determine alkanolamines have included wet chemical techniques, gas chromatography, thin-layer chromatography, and high-performance liquid and ion chromatography (HPLC, HPIC) coupled with spectrometric, electrochemical or conductivity detection. Wet chemical techniques generally are more precise than instrumental methods; however, their application to complex real-life matrices is made difficult by the need to isolate the analytes from the sample matrix. Gas chromatography is possible [4–8], but the high polarity of alkanolamines makes them difficult to analyze in this manner. Thin-layer chromatography with photometric detection has been demonstrated for the determination of β-alkanolamines [9]. However, because of the absence of natural chromophores and/or fluorophores, photometric detection of alkanolamines requires they be

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derivatized with a spectrometrically active adduct. The same is true for the HPLC techniques utilizing photometric detection [10–14]. Derivatization techniques can be time-consuming, and quantitation sometimes made difficult because the alkanolamines are derivatized with varying efficiencies in different sample matrices. Therefore, direct detection is preferable whenever possible. Conductivity detection has been used for the direct detection of alkanolamines separated by HPIC [15–17]; however, the sensitivity of conductivity detection is generally not as good as that of most other chromatographic detection methods.

Recently, pulsed electrochemical detection (PED) coupled with HPLC has been shown to be a viable method for the determination of alkanolamines. LaCourse et al. [18] demonstrated the separation of mono-, di- and trialkanolamines using a dodecanesulfonate ion-pair reagent and a silica-based C_{18} reversed-phase column. Although determination of alkanolamines was possible at the ppb level, relatively long chromatographic runs (1–2 h) were necessary to obtain reasonable separation. Campbell et al. [2] also determined alkanolamines via HPLC–PED, employing the reversed-phase characteristics of a polymer-based column (Dionex PAX-500) to separate diethanolamine (DEA) and triethanolamine (TEA) in an aluminum etching bath. However, use of the same column in our laboratory indicated that the smaller and more hydrophilic alkanolamines were not strongly retained, and often were unresolved from each other and from the solvent peak.

Because of the hydrophilic nature of aliphatic alkanolamines, HPIC seemed to hold greater promise than reversed-phase HPLC as a separation technique for these compounds. However, a column that combines both cation-exchange and reversed-phase retention properties (Dionex PCX-500) might be expected to hold advantages for alkanolamine separations. A preliminary demonstration of HPLC–PED using this type of column was included in a recent review [19]. Herein we provide a more complete description of alkanolamine retention on a so-called "multimodal" column. The separation of alkanolamines is studied as a function of mobile phase composition, with retention measured versus both the counter cation (Na^+) and organic modifier (acetonitrile, ACN) concentration in the eluent. Using optimized isocratic conditions, baseline resolution is shown for a mixture of alkanolamines, including positional isomers. The method is also demonstrated for the determination of alkanolamines in a commercial formulation and a biological sample.

2. Experimental

2.1. Reagents

All chemicals used in preparation of chromatographic eluents were reagent grade or better and used as received. Sodium acetate (Fisher) was obtained in either anhydrous or trihydrate form. Glacial acetic acid (Fisher) and acetonitrile (Fisher) were HPLC grade. All mobile phases were filtered through a 0.2-μm nylon filter (Whatman) prior to use. The 0.30 *M* solution of NaOH mixed with the chromatographic effluent stream was prepared by dilution of a commercially available 50% (w/w) solution (Fisher).

Ethanolamine (Fisher) and all other alkanolamines (Aldrich) were of the best grade available. Perchloric acid (Fisher) used for dilution of biological samples was reagent grade. Water for all solutions was purified by passing tap water through two D-45 deionizing tanks (Culligan) and a Milli-Q system (Millipore).

2.2. Voltammetric apparatus and procedures

Pulsed voltammetry was performed at the gold disk of an AFMT28AUAU rotating ring-disk electrode (RRDE, Pine Instruments). Rotation of the electrode was provided by an AFMSR rotator (Pine). The counter electrode for the electrochemical cell was provided by a coiled platinum wire. Potentials are reported versus a saturated calomel electrode (SCE, Fisher). The electrochemical cell was made of Pyrex, and had porous glass frits separating the compartments for the working, reference and counter elec-

trodes. Potential control was maintained with an AFRDE4 bi-potentiostat (Pine) interfaced to a personal computer (PC, Jameco) via a DT2801-A data acquisition board (Data Translation). Potential–time waveforms were generated by the PC using programs written in ASYST 4.0 software (Keithley/Asyst). Electrolyte solutions were deaerated by dispersed nitrogen gas.

2.3. Chromatographic apparatus and procedures

Unless noted otherwise, all chromatographic equipment was from Dionex. Separations utilized either a full-sized (250 × 4 mm) or guard (50 × 4 mm) version of the PCX-500 column. Sample injection was provided by a pneumatically activated injector equipped with a 25-μl sample loop. A GPM gradient pump and pulsed electrochemical detector were interfaced to a Zenith PC through an AI-450 chromatography automation system.

The Dionex PED system provides the options of obtaining an output signal corresponding to: (i) the time-integral of electrode current over the integration period of the waveform ($\int i\partial t$, C) or (ii) the average current with the integration period ($\int i\partial t/t_{INT}$, C s^{-1}). The first option was selected in this research. PED was performed in a flow-through cell consisting of a 1.4-mm diameter Au working electrode and a Ag/AgCl reference electrode. The counter electrode was provided by the upper half of the detection cell, which was made of stainless steel. A solution of 0.30 *M* NaOH was added post-column through a mixing tee, with constant flow maintained by a post-column pneumatic controller. The post-column eluent had a final pH of ca. 13, providing the alkaline environment necessary for alkanolamine detection at the Au working electrode.

3. Results and discussion

3.1. Voltammetry of alkanolamines

Selection of the optimal detection potential for use in HPLC–PED was based on current–potential (i–E) curves obtained by pulsed voltammetry. Typical results are shown in Fig. 1 for tris(hydroxymethyl)aminomethane (Tris). Use of pulsed voltammetry for optimization of PED waveforms has been described [20]. The data in Fig. 1 were obtained by positive scan of the detection potential (E_{DET}) according to a staircase waveform consisting of 10-mV step increments, while maintaining the oxidative cleaning potential (E_{OXD}) and reductive regeneration potential (E_{RED}) at constant value of +0.80 V and −0.80 V, respectively. Values of electrode current shown in Fig. 1 correspond to average values for an integration period (t_{INT}) of 50 ms following a delay period (t_{DEL}) of 250 ms during the detection period ($t_{DET} = t_{DEL} + t_{INT}$) for each application of the PED waveform.

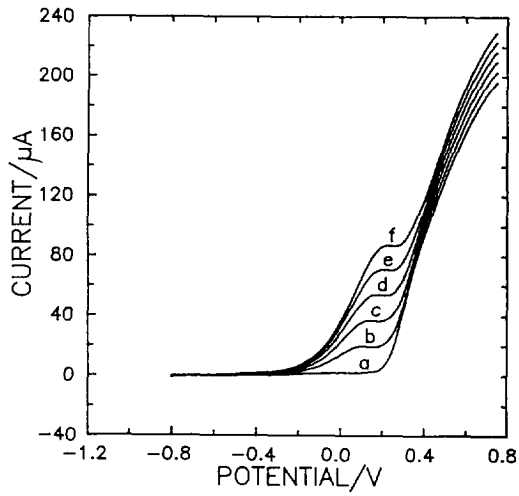

Fig. 1. Pulsed voltammetric response of Tris at a Au RDE in 0.1 *M* NaOH. Rotation rate: 400 rpm. Potential waveform: E_{DET} in the PED waveform is scanned from −0.80 V to 0.75 V according to a staircase waveform using 10-mV step increments (t_{DET} = 300 ms, t_{DEL} = 250 ms); E_{OXD} = 0.80 V (t_{OXD} = 120 ms); E_{RED} = −0.80 V (t_{RED} = 380 ms). Curves: a = 0 μM; b = 20 μM; c = 40 μM; d = 60 μM; e = 80 μM; f = 100 μM Tris.

In the absence of Tris (curve a), most of the current generated by pulsed voltammetry at the Au working electrode is the result of formation of surface oxide (AuO) at $E_{DET} > 0.2$ V. Anodic response for Tris (curves b–f) is obtained for $E_{DET} >$ ca. −0.2 V with a plateau signal for E_{DET} = 0.0 to 0.2 V (e.g., curve b) at low concentrations and 0.1 to 0.2 V at high con-

centrations (e.g., curve f). In this plateau region, the PED response to Tris appears to be a linear function of concentration, which is indicative of a mass-transport limited reaction. A similar response was obtained for all other alkanolamines studied, including 2-amino-1-ethanol (ETH), 3-amino-1-propanol (PRO), 4-amino-1-butanol (BUT) and 5-amino-1-pentanol (PEN). Based on these results the maximum signal-to-background ratio (S/B) is obtained for E_{DET} in the range 0.0 to 0.1 V.

3.2. Optimization of HPLC conditions

A preliminary separation of five alkanolamines is shown in Fig. 2 for the PCX-500 (250 × 4 mm) column. The mobile phase consisted of an acetate buffer (pH ≈ 5) which is sufficiently acidic to ensure protonation of the amine group, thereby producing the cationic form of the alkanolamines necessary for retention on the multimodal column. Because alkanolamines are not oxidized at Au electrodes in acidic media, post-column addition of NaOH was used to provide the alkaline environment necessary to obtain PED response. Sodium acetate (NaOAc) in the eluent provided Na^+ as the counter ("pusher") ion necessary to elute the alkanolamines from the cation-exchange portion of multimodal column. In general, retention time increases as a function of decreasing hydrophilicity of the cationic alkanolamines. Tris, with three alcohol groups, is the most hydrophilic and, therefore, exhibits the least retention. With the exception of ETH and PRO, baseline resolution was possible for the terminal amino alcohols under the conditions used for Fig. 2. The small peak at ca. 2 min is the result of O_2 dissolved in the sample which is reduced to H_2O_2 at E_{RED} and subsequently detected anodically by oxidation to H_2O at E_{DET}.

Fig. 3 shows the retention factors (k) for five alkanolamines plotted versus the concentration of ACN in the acetate mobile phase. It is evident that the retention factors for the larger and more hydrophobic alkanolamines, e.g., BUT (d) and PEN (e), are most affected by the amount of ACN in the mobile phase. This is to be expected

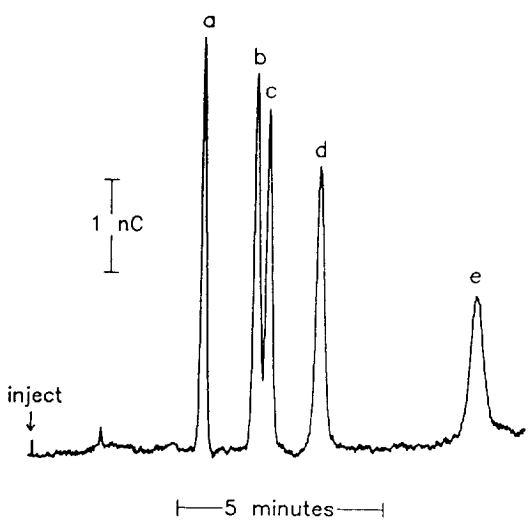

Fig. 2. HPLC–PED of five alkanolamines. Column: Dionex PCX-500 (250 × 4 mm). Injection: 25 μl. Eluent: 20 m*M* HOAc/60 m*M* NaOAc at 1.0 ml min $^{-1}$. Post-column addition: 0.30 *M* NaOH at 0.6 ml min $^{-1}$. PED waveform: E_{DET1} = 0.05 V (t_{DET} = 300 ms, t_{DET} = 250 ms); E_{OXD} = 0.80 V (t_{OXD} = 120 ms); E_{RED} = −0.40 V (t_{RED} = 180 ms). Peaks: a = 4 μ*M* Tris; b = 10 μ*M* ETH; c = 10 μ*M* PRO; d = 10 μ*M* BUT; e = 10 μ*M* PEN.

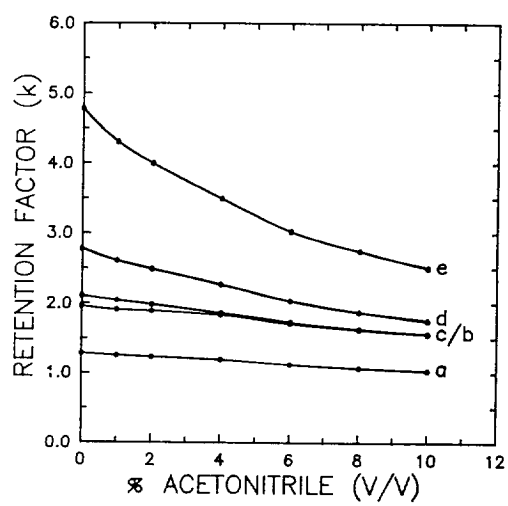

Fig. 3. Retention factors (k) plotted versus eluent acetonitrile (ACN) content. Column: Dionex PCX-500 (250 × 4 mm). Eluent: 20 m*M* HOAc/60 m*M* NaOAc–variable ACN at 1.0 ml min $^{-1}$. Post-column addition: 0.30 *M* NaOH at 0.6 ml min $^{-1}$. PED waveform as in Fig. 2. Curves: a = Tris; b = ETH; c = PRO; d = BUT; e = PEN.

for a retention mechanism having a significant reversed-phase component. The ACN concentration, while not having a strong influence on the retention of the smaller alkanolamines, is critical to the separation of ETH and PRO (b and c). For ACN concentrations larger than 2% (v/v), the retention factors for ETH and PRO are virtually identical. Hence, the best separation of these two compounds is obtained without ACN present in the eluent. Although literature supplied with the column indicates that the use of mobile phases containing no organic solvent may damage the column, we saw no indication of column deterioration over several months of operation. Larger alkanolamines ($>C_6$) also can be eluted using this separation strategy. However, as the hydrophobicity of the alkanolamine increases, there is an increasing need for the addition of organic modifier to the mobile phase. For example, to obtain reasonable elution times for the C_6–C_8 alkanolamines on the 250- mm column, a mobile phase containing between 5–15% ACN is suggested.

Fig. 4 shows values of the retention factors for alkanolamines versus the concentration of Na^+, added as NaOAc, in the mobile phase without the presence of ACN. These results demonstrate a significant decrease in k with increasing Na^+ concentration, indicating a strong cation-exchange component for the retention of alkanolamines on the multimodal column. The optimum separation of alkanolamines is realized at NaOAc eluent concentrations in the range 40–100 mM. Variation of the acetic acid (HOAc) concentration in the mobile phase had little effect on alkanolamine retention, providing there was sufficient HOAc (>10 mM) to assure protonation of the amine functional groups.

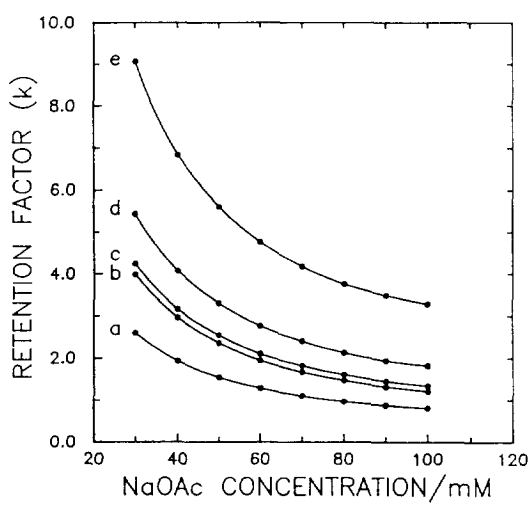

Fig. 4. Retention factors (k) plotted versus eluent NaOAc content. Column: Dionex PCX-500 (250 × 4 mm). Eluent: 20 mM HOAc/variable NaOAC at 1.0 ml min^{-1}. Post-column addition: 0.30 M NaOH at 0.6 ml min^{-1}. PED waveform as in Fig. 2. Curves: a = Tris; b = ETH; c = PRO; d = BUT; e = PEN.

3.3. Detection limits, linearity and reproducibility

The linear dynamic range of the HPLC–PED response for alkanolamines was determined using Tris as a representative compound. Response was linear within the range 50 nM to 100 μM, as indicated by the following regression statistics: slope = 1.57 nC μM^{-1}, intercept = 0.17 nC, and correlation coefficient (R^2) = 0.9997 (n = 11). The limit of detection (S/N = 3) for 25-μl injections of Tris is estimated to be 20 nM (500 fmol). Negative deviations from linear response were observed beginning at concentrations greater than 200 μM and, for concentrations above 2000 μM, the PED signal changed only slightly with increased concentration. These calibration data were obtained using E_{DET} = 0.0 V to minimize the background signal and obtained the lowest possible detection limit. Examination of the pulsed voltammetric data in Fig. 1 reveals that this detection potential does not correspond to the plateau response for high concentrations of Tris. Therefore, choice of E_{DET} = 0.1 V is recommended to achieve maximum linearity of calibration curves, albeit with some sacrifice of detectability.

Fig. 5 shows HPLC–PED results for ten consecutive injections of 10 μM Tris. The relative standard deviation (R.S.D.) of these peak heights is ca. 0.4%. This indicates that HPLC–PED provides a very reproducible response for alkanolamines.

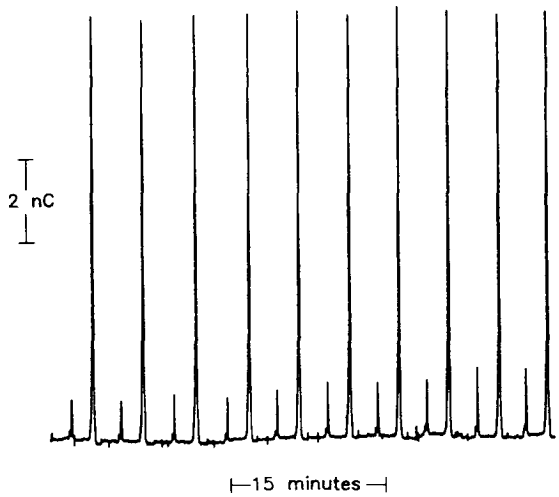

Fig. 5. Reproducibility of HPLC response. Column: Dionex PCX-500 (250 × 4 mm). Injections: 25 μl of 10 μ*M* Tris. Eluent: 20 m*M* HOAc/60 m*M* NaOAc at 1.0 ml min^{-1}. Post-column addition: 0.30 *M* NaOH at 0.6 ml min^{-1}. PED waveform as in Fig. 2.

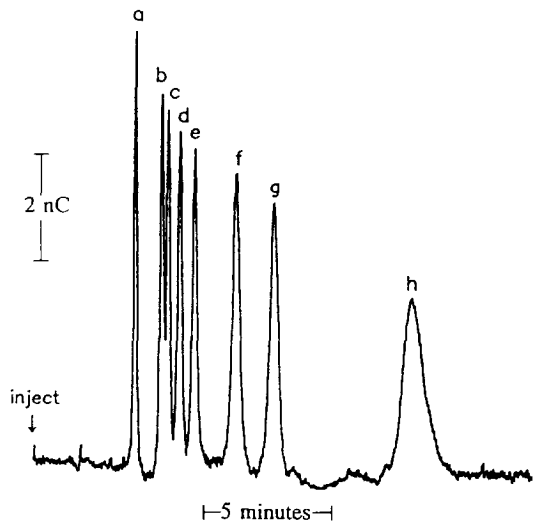

Fig. 6. HPLC–PED of alkanolamine isomers. Column: Dionex PCX-500 (250 × 4 mm). Injection: 25 μl. Eluent: 20 m*M* HOAc/70 m*M* NaOAc at 1.0 ml min^{-1}. Post-column addition: 0.30 *M* NaOH at 0.6 ml min^{-1}. PED waveform as in Fig. 2. Peaks: a = 8 μ*M* Tris; b = 20 μ*M* ETH; c = 20 μ*M* PRO; d = 20 μ*M* 2-amino-1-propanol; e = 40 μ*M* BUT; f = 40 μ*M* 2-amino-1-butanol; g = 40 μ*M* PEN; h = 80 μ*M* 2-amino-1-pentanol.

3.4. Isomeric separation and other applications

The separation of several alkanolamine positional isomers is demonstrated in Fig. 6. Compare peaks c and d for PRO and 2-amino-1-propanol, respectively; peaks e and f for BUT and 2-amino-1-butanol, respectively; and peaks g and h for PEN and 2-amino-1-pentanol, respectively. It is apparent that alkanolamine isomers with terminal amine groups are eluted before their β-amino analogues. This order of elution is explained on the basis of the relative positions of the functional groups. β-Alkanolamines have both the amine and the alcohol functional groups near one end of the molecule. As a result, a larger portion of the molecular structure is hydrophobic, resulting in stronger retention on the reversed-phase portion of the multimodal column. Furthermore, variation of the acetonitrile concentration in the mobile phase had a greater effect on the retention of the β-amino alcohols than the retention of the terminal-amino alcohols, which supports the conclusion that the β-alkanolamines are more hydrophobic.

Application of the HPLC–PED system for the determination of an alkanolamine in a commercial shaving gel using a 50 × 4 mm column is shown in Fig. 7. Minimal sample preparation was required, consisting of dilution (1:10 000, w/v) of a small portion of the sample with deionized water before injection. The resulting chromatogram exhibits only two peaks, and its simplicity is largely attributable to the choice of E_{DET} in the PED waveform. At $E_{DET} = 0.05$ V, PED is fairly selective for organic compounds with the alcohol moiety. Thus, many possible interferents show no response at the Au working electrode. The first peak is from sorbitol, an ingredient in the shaving gel. The second peak corresponds to TEA. The relative standard deviation for five determinations of TEA was better than 1.0%, thus indicating that even with very little sample preparation, good precision is possible. The shoulder on peak b for TEA suggest an overlap of two unresolved peaks. However, a second compound was never resolved from TEA using this column or the longer analytical column

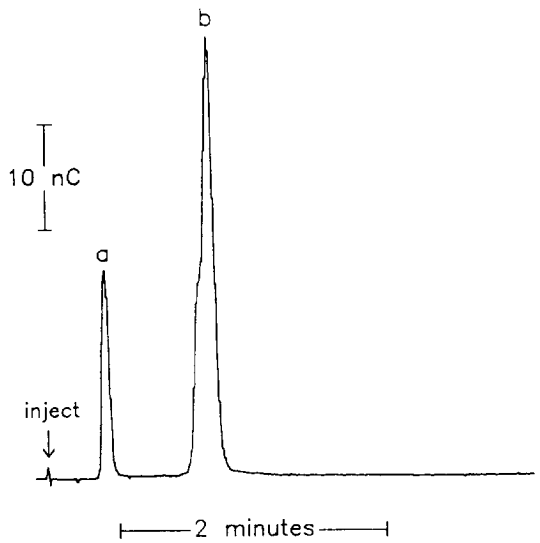

Fig. 7. HPLC–PED of commercial shaving gel. Column: Dionex PCX-500 (50 × 4 mm). Injection: 25 μl. Eluent: 20 m*M* HOAc/60 m*M* NaOAc at 1.0 ml min^{-1}. Post-column addition: 0.30 *M* NaOH at 0.6 ml min^{-1}. PED waveform as in Fig. 2. Peaks: a = sorbitol; b = TEA.

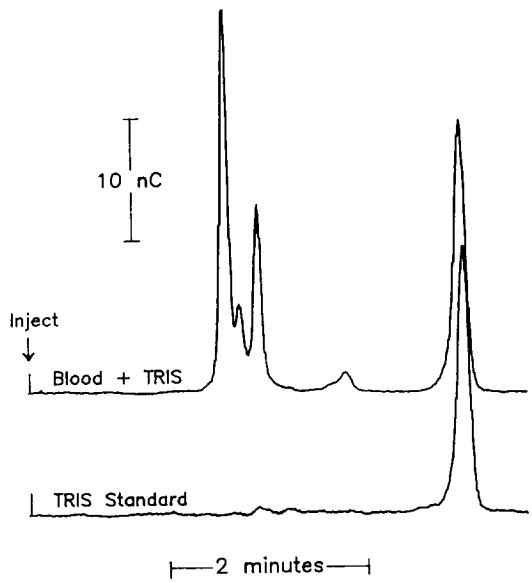

Fig. 8. HPLC–PED of Tris in blood serum. Column: Dionex PCX-500 (250 × 4 mm). Injections: 25 μl. Eluent: 20 m*M* HOAc/60 m*M* NaOAc at 1.0 ml min^{-1}. Post-column addition: 0.30 *M* NaOH at 0.6 ml min^{-1}. PED waveform as in Fig. 2. Upper chromatogram: serum from human blood spiked with Tris and diluted with deionized water to a final concentration of 20 μ*M*. Lower chromatogram: 20 μ*M* Tris standard.

(250 × 4 mm). The cause of the anomalous peak shape in Fig. 7 is unknown at the present time.

The HPLC–PED system was tested for determination of Tris in human blood serum. Tris is known clinically as Tromethamine and is used as a blood buffering agent [21]. Furthermore, Arispe et al. [22] recently reported that Tris has possible therapeutic value in the treatment of Alzheimer's disease. Therefore, the clinical determination of Tris in blood might be of some interest. Artificial samples of Tris in blood were prepared as follows: 100-μl aliquots of human blood were added to 100-μl aliquots of 2.00 m*M* Tris. These mixtures then were diluted to 10.0 ml either with deionized water or with 1.0 *M* $HClO_4$ to give a final Tris concentration of 20.0 μ*M*. The solutions were mixed thoroughly, centrifuged, and filtered through a 0.22-μm syringe filter (Whatman) to remove red blood cells prior to injection.

The HPLC–PED results obtained for the blood samples containing 20.0 μ*M* Tris are adequately represented by the upper chromatogram in Fig. 8 for a sample diluted with water. The lower chromatogram represents the response for a 20.0 μ*M* Tris standard in water. The recovery efficiency for Tris in blood was dependent upon the sample diluent. When samples were diluted with deionized water, recovery efficiency was 81.6 ± 3.1% ($n = 4$). When 1.0 *M* perchloric acid was the diluent, the recovery efficiency was 102.0 ± 2.3% ($n = 4$). We speculate that the lower recovery efficiency for samples diluted with water is the result of complexation of Tris by blood proteins resulting in a decreased concentration of free Tris. Dilution of samples with acid caused the proteins to be ionized, thus releasing the bound Tris and allowing complete recovery. Another benefit of using acid as the blood diluent relates to sample stability. Injections could be made several days after sample dilution by acid without any discernible decrease in the recovery efficiency. Other peaks in the upper chromatogram have

not been identified, with the exception of the first peak that corresponds to normal levels of blood sugar (glucose).

4. Conclusions

The separation of alkanolamines was demonstrate using HPLC. The separation relies upon both cation-exchange and reversed-phase retention mechanisms of a multimodal column, and the strategy provides baseline resolution of positional isomers of several alkanolamines using isocratic elution. When coupled with PED, the determination of alkanolamines was shown to be sensitive and reproducible. Detection limits in the nanomolar region were possible, and the linear dynamic range was greater than three decades. The method has the advantage of being rugged, with minimal sample preparation required to successfully analyze alkanolamines in both commercial and biological samples.

Acknowledgement

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