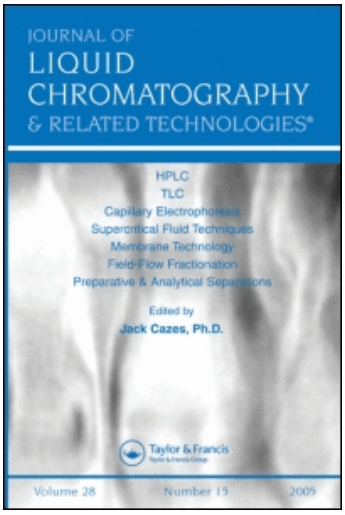

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Determination of Suttocide®A in Cosmetic Formulations by Single Column Ion Chromatography with Pulsed Amperometric Detection (SCIC-PAD)

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DETERMINATION OF SUTTOCIDE®A IN COSMETIC FORMULATIONS BY SINGLE COLUMN ION CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (SCIC-PAD)

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ABSTRACT

Single column anion chromatography is used for the determination of Suttocide®A in shampoo, styling gel, conditioner, and skin moisturizer formulations. After quantitative decomposition of the analyte, the resulting sodium glycinate is separated on a polymeric anion exchange column, using a mobile phase of 0.15 M NaOH. Specific detection is accomplished using integrated amperometry at a gold electrode. Inorganic anions as well as other formulation ingredients do not interfere under the analytical conditions, allowing for a detection limit of 50 ppm Suttocide®A in the samples.

INTRODUCTION

Chemical compounds that inhibit bacterial and mold growth are commonly employed in cosmetic formulations. These preservatives must

not only be effective at relatively low concentrations, but also be safe and non-irritating during use. Shampoos and conditioners (particularly those containing hydrolyzed animal protein) can be particularly prone to contamination and require preservative systems that are active over a broad pH range.

A newly developed antimicrobial preservative that meets the above requirements is known as Suttocide®A (1). The active component sodium hydroxymethylglycinate, $HO\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}COO^-Na^+$), sold as a 50% aqueous solution, has been shown to have broad spectrum activity against Gram-positive and Gram-negative bacteria as well as yeast and mold. It is somewhat unique in that it maintains its activity at elevated pH. Numerous toxicological tests have confirmed the safety of Suttocide®A (2). In addition, its high water solubility eliminates partitioning into the oil phase of emulsion formulations.

Quality control of the formulation process requires methods of quantitation for all components, including the preservative system. In general, the complexity of typical formulations requires extensive sample cleanup to insure accuracy and specificity. It is desirable to utilize a minimum of cleanup steps to minimize losses. Suttocide®A poses a challenge to the analyst as it contains neither a UV absorbing nor a

fluorescing chromophore, making sensitive detection by HPLC impossible. In addition, the compound's amine methylol structure may be unstable to conditions used for common analytical procedures. This necessitates the use of quantitative decomposition with subsequent analysis of the decomposition products.

We have developed a highly specific method for the determination of Suttocide®A in a variety of cosmetic formulations. In general, the diluted sample is heated to an elevated temperature which generates sodium glycinate as a decomposition product. Sodium glycinate is then separated from the matrix by single column anion chromatography using a mobile phase of 0.15 M NaOH. Integrated pulsed amperometric detection at a gold electrode yields specific and sensitive response for sodium glycinate, allowing for quantitative analysis of Suttocide®A. Under the conditions of analysis, detection limit of 50 ppm is obtained with a minimum of sample cleanup.

EXPERIMENTAL

Apparatus

Voltammetric data were obtained at a gold rotated disk electrode (AuRDE) (Model RDE-1, Bioanalytical Systems, West Lafayette, Indiana)

connected to a BAS 100 Electrochemical Analyzer. All voltammetric experiments utilized a three electrode configuration (AuRDE = working electrode; Ag/AgCl, saturated KCl = reference electrode; Pt wire = counter electrode) with rotation speed of 1000 rpm and scan rate of 100 mV/s. A Houston Instruments HI-PLOT X-Y digital plotter was used to record current - potential curves. All voltammograms were obtained on solutions after degassing with O_2 - free argon for 10 minutes.

Liquid chromatographic separations were obtained in the isocratic mode using a Hamilton PRP-X100 polymeric anion exchange column (4.6 mm x 250 mm) and PRP-X100 guard column. An Applied Biosystems 400 Solvent Delivery Pump, SSI pulse dampener, Waters 715 WISP Autoinjector, Dionex Pulsed Electrochemical Detector (PED), and in-house Hewlett Packard data acquisition system (HP1000) completed the chromatographic setup.

The PED was operated exclusively in the integrated pulsed amperometry (IPAD) mode. The cell was of the thin layer design (Dionex) and was composed of a gold working electrode, a glass pH/combination reference electrode, and a stainless steel counter electrode. The working electrode was polished sequentially with 6 μm diamond paste and 0.05 μm alumina followed by sonication in HPLC

grade water before use. The pH reference electrode was calibrated using pH 7.00 and pH 10.00 buffer solutions (VWR Scientific, Piscataway, NJ).

A Kontes Ultraware Glass Filtration Apparatus with 0.45 μm Nylon 66 filter membranes (47 mm diameter, Alltech Associates, Deerfield, Illinois) was used to filter the mobile phase prior to use. Maxi - Clean 300 IC-Ba^{2+} cartridges (Alltech) or 0.45 μm glass fiber filtration cartridges (Whatman, Inc., Clifton, NJ) were used for sample cleanup, depending on the nature of the formulation, prior to analysis. A Reacti-Therm Heating/Stirring Module (Pierce Chemical Co., Rockford, Illinois) was used for analyte decomposition.

Reagents

HPLC grade water (OmniSolv) and NaOH pellets (analytical reagent) were obtained from EM Science. Glycine (99+%) was obtained from Aldrich Chemical Co. A stock solution was prepared by dissolving the material in water, adjusting to pH 11.5 with 1 N NaOH, and diluting to volume. This solution was prepared fresh daily. Suttocide®A (50% (w/w) solution), obtained from Sutton Laboratories, Chatham, NJ., was assayed by determination of the total nitrogen content via Kjeldahl titration.

Procedure

Sample solutions were prepared by diluting an appropriate quantity with HPLC grade water. Diluted solutions of shampoo and conditioner formulations were first treated with Ba^{2+} cartridges to remove excess sulfate. This was accomplished by passing ca. 4 mL of solution through the cartridge and collecting the last 3 mL. All other standards and diluted formulations were simply filtered through 0.45 μm glass fiber filter cartridges. The collected solutions were then heated at 60° C. ± 1° C. for 15 minutes and cooled to room temperature before injection.

The following chromatographic conditions were employed for all analyses:

(a) Column: Hamilton PRP X100, 4.6 mm x 250 mm
Hamilton PRP X100 guard column

(b) Mobile phase: 0.15 M NaOH

(c) Flow rate: 1 mL/min

(d) Inj. vol.: 25 μL

(e) Detection: PED, 3 μC full scale, 10 mV output

The potential pulse sequence which yielded optimum response (best signal to noise ratio) is shown in Table I. Current, integrated during the 0.20 s to 0.70 s time period, was output to the data acquisition system

TABLE I

Potential Pulse Sequence for Au Electrode

Time, seconds	Volts vs. pH ref.
0.00	0.00
0.20	0.00
0.30	0.60
0.40	0.60
0.50	0.00
0.70	0.00
0.71	0.80
0.90	0.80
0.91	0.00
1.00	0.00

to generate analyte peak response. The baseline was allowed to stabilize at a flow rate of 1 mL/min for 30 minutes before beginning the chromatographic analysis.

RESULTS AND DISCUSSION

The determination of aliphatic amine, amino acid, aldehyde, and hydroxyl - containing compounds has historically been accomplished using derivatization and HPLC approaches (3). While generally accepted, this complicates the analysis of formulated products and can lead to poor recovery due to increased sample handling. Johnson, et. al.

(4,5) have pioneered the use of HPLC with pulsed amperometry at noble metal electrodes for the direct detection of these compounds. As Suttocide®A and its decomposition products contain all of the above mentioned functionalities, it was critical to define the electrochemical behavior of all species so that the optimal analytical scheme could be designed.

An excellent diagnostic tool for the determination of electrochemical behavior under hydrodynamic (convective transport) conditions is cyclic voltammetry at the rotating disk electrode. This experiment allows one to rapidly observe the oxidation-reduction behavior of species under simulated chromatographic flow conditions. Figure 1 displays the typical voltammogram of the gold electrode in deoxygenated 0.15 M NaOH. The anodic scan shows the formation of an oxide layer (+0.1 V to +0.6 V) while the reverse scan results in the cathodic dissolution of the oxide (+0.2 V to -0.2 V). The peak currents and potential are highly reproducible with multiple scans. Suttocide®A and formaldehyde show complex electrochemical behavior at the gold electrode in the same electrolyte. For Suttocide®A, (Figure 2) an oxidation process takes place at ca. -0.40 V which overlaps a diffusion controlled oxidation at more anodic potential (-0.30 V to +0.20 V). The

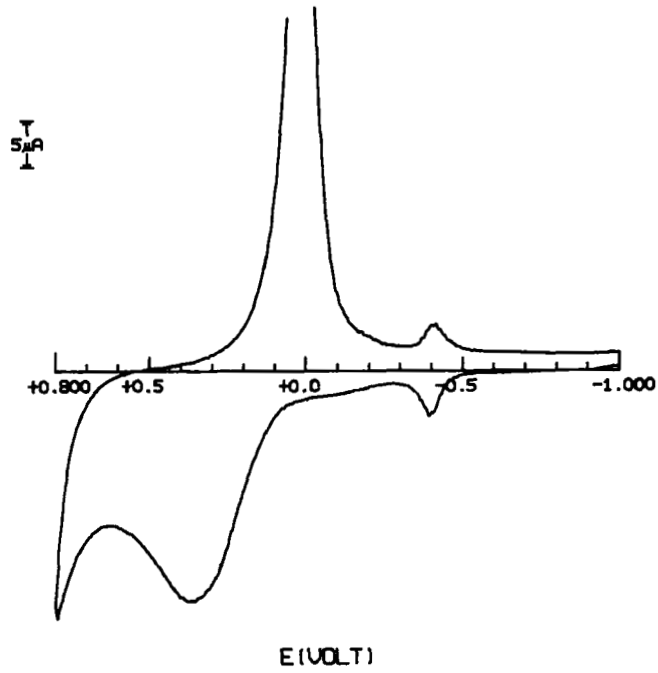

FIGURE 1. Cyclic voltammogram of 0.15 M NaOH at the gold rotating disk electrode (AuRDE). 1000 rpm; 100 mV/s.

latter oxidation is similar to that observed for formaldehyde (Figure 3). A cathodic shift of the oxide layer formation process is also observed which suggests adsorption of the molecule on the electrode surface. This was confirmed by performing multiple scans (n = 10; not shown) and observing an increase in the oxidation peak current at -0.40 V with each successive scan.

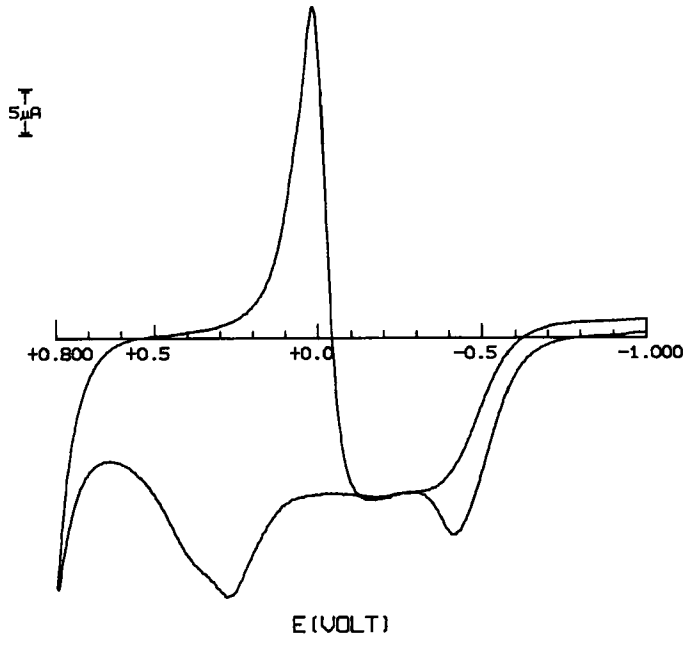

FIGURE 2. Cyclic voltammogram of 4 mM Suttocide®A in 0.15 M NaOH at the AuRDE. 1000 rpm; 100 mV/s.

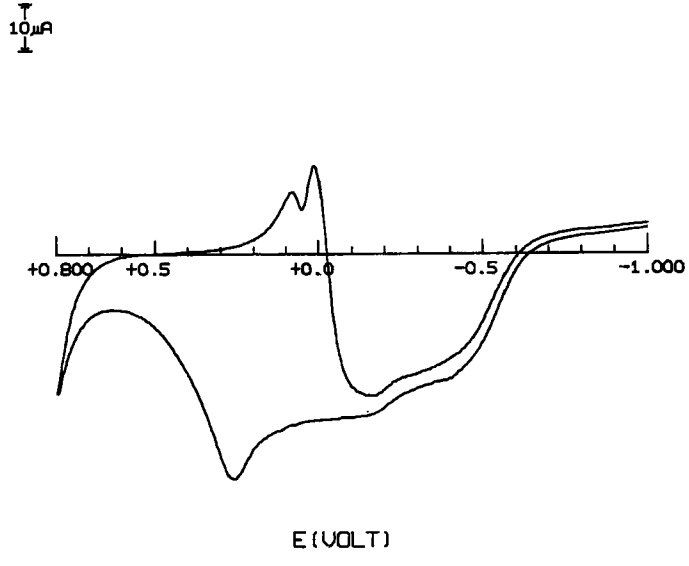

FIGURE 3. Cyclic voltammogram of 1 mM formaldehyde in 0.15 M NaOH at the AuRDE. 1000 rpm; 100 mV/s.

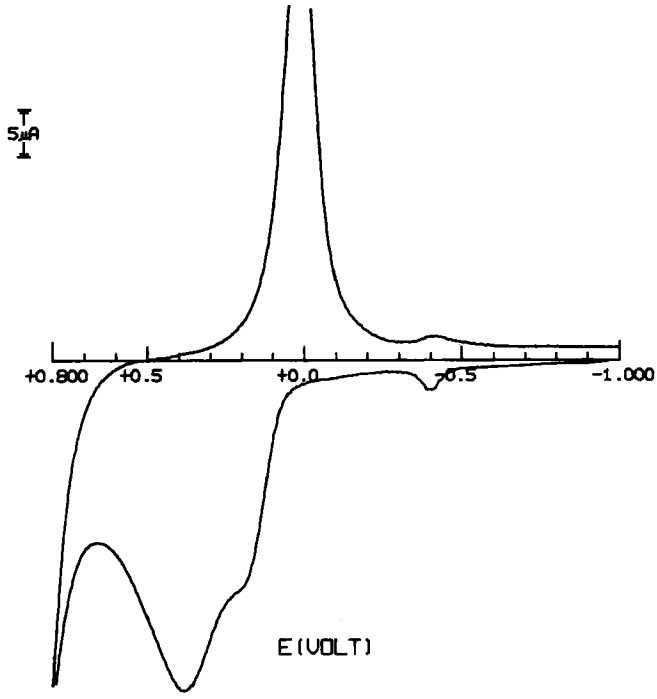

FIGURE 4. Cyclic voltammogram of 4 mM sodium glycinate in 0.15 M NaOH at the AuRDE. 1000 rpm; 100 mV/s.

While the dissolution process for the oxide layer is similar for the blank electrolyte and Suttocide® A, a significant decrease in peak current and shape is observed for this process in the presence of formaldehyde. This strongly suggests a passivation of the electrode surface by formaldehyde or its oxidation products at the electrode surface. The electrochemical behavior of sodium glycinate (Figure 4) is

consistent with that reported for aliphatic amino acids at the gold electrode (5). An oxide catalyzed oxidation is observed in the region +0.10 V to +0.60 V which is amenable to the integrated pulsed amperometric detection scheme. A triple pulse waveform allows for detection, cleaning, and oxide renewal under flow conditions. Because of the complicated electrochemistry of Suttocide®A at the gold electrode as well as the difficulty of developing suitable chromatographic retention for a variety of supports and mobile phases, it was decided develop an analytical procedure based on quantitative decomposition of dilute Suttocide®A to sodium glycinate followed by anion exchange chromatography and integrated pulsed amperometric detection.

Ion exchange separation of amino acids can be accomplished by either anion or cation exchange techniques. For the present study, anion exchange provided the most straightforward situation. As the pK_a of glycine is 9.78 (6), elevated pH will completely ionize the carboxylic acid group, allowing for favorable anion exchange conditions. While hydroxide eluants have weak elution power in SCIC, the retention time of glycine can be adjusted by proper selection of the NaOH concentration. In addition, the hydroxide eluant is well matched for pulsed amperometric detection at the gold electrode without the need

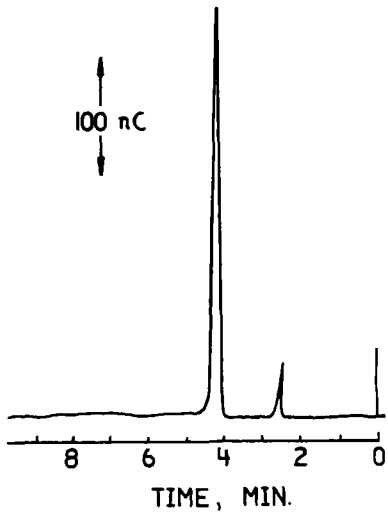

FIGURE 5. Chromatogram of 9.2 ppm sodium glycinate. Conditions given in text.

for post-column addition of base. The potential pulse waveform given in the previous section was optimized for best peak shape. It should be noted that the use of the pH reference electrode necessitated increasing all applied potentials by ca. 300 mV due to the dependence of potential on pH (-59 mV/pH unit). This has been reported previously by Johnson, et al. (4,5).

A typical chromatogram of sodium glycinate is shown in Figure 5. Under the experimental conditions, sodium glycinate elutes in 4.0 minutes (k' = 1) while formaldehyde is not retained on the column.

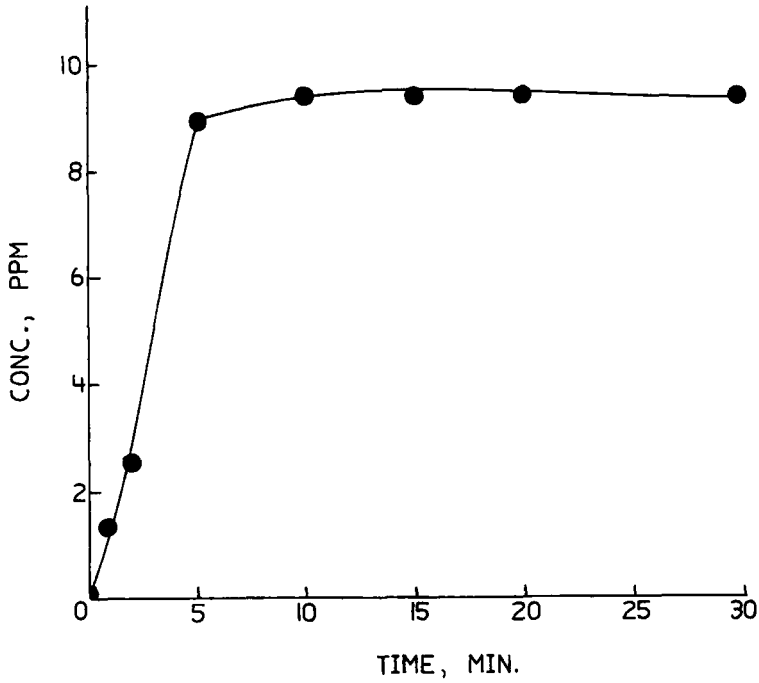

FIGURE 6. Decomposition of 10 ppm Suttocide®A at 60° C. with time.

Dilute Suttocide®A is quantitatively decomposed to sodium glycinate by heat. Figure 6 indicates that more than 90% decomposition takes place at 60° C. after 10 minutes without further change at longer contact times. Thus, 15 minutes was chosen to insure maximum conversion at a reasonable heating time. It should be noted that the decomposition behavior is characteristic of only highly dilute solutions ($\leq$ 100 ppm). Suttocide®A is thermally stable at the levels typically used in

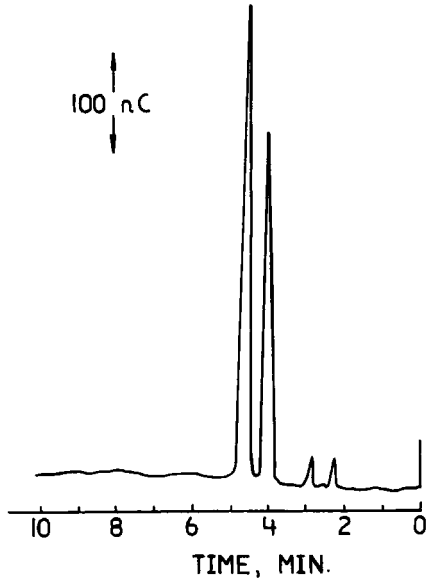

FIGURE 7. Chromatogram of 0.50 g shampoo formulation in deionized water containing 0.18% Suttocide®A. Sample subjected to cleanup and heating as described in the text. Peak at 4 minutes is sodium glycinate.

formulations (0.1% - 0.5%) as well as in the raw material form (50%). While common inorganic anions (eg. chloride, sulfate) do not interfere as they have no electrochemical activity, large excesses of sulfate (which can be present in shampoos and conditioners) must be removed to prevent column overload and loss of chromatographic efficiency. Shown in Figure 7 is a typical chromatogram of a shampoo formulation containing 0.18% Suttocide®A. Although other species appear to be electroactive at the gold electrode, sodium glycinate is resolved from

other components in the matrix allowing for quantitation using peak height measurement and external standards.

Response linearity was evaluated in the 0.5 ppm to 30 ppm sodium glycinate range. A plot of peak height versus concentration was linear and obeyed the following equation:

$$H(nC) = (36.6 \pm 1.3 \text{ nC/ppm})\ C + 2.8 \pm 2.0 \text{ nC}$$

The correlation coefficient and standard error of estimate are 0.998 and 2.54, respectively. Concentrations in excess of 50 ppm were found to deviate from linearity. This is typical of anodic response at noble metal electrodes and has been reported previously (7). The detection limit, defined as the sodium glycinate concentration yielding a signal to noise ratio of 3, is 0.2 ppm. This corresponds to a Suttocide®A concentration of 0.26 ppm in solution. Depending on sample size and injection volume, the detection limit in formulated samples is typically 50 ppm.

Method precision was evaluated by both the multiple injection (n = 8) and multiple sample (n = 8) technique. Replicate injections of a 13.34 ppm sodium glycinate standard solution had an average peak height of 515 nC $\pm$ 9.5 nC at the 95% confidence level. The coefficient

TABLE II

Recovery of Suttocide®A from Formulations

Sample	Added,%	Found,%	Recovery,%
Shampoo	0.05	0.052	104
	0.10	0.099	99
	0.15	0.151	101
Conditioner	0.05	0.049	98
	0.10	0.098	98
	0.15	0.143	95
Styling gel	0.50	0.45	90
	0.50	0.43	86
Moisturizing	0.35	0.30	86
Facial	0.35	0.29	83
Treatment	0.35	0.28	80
	0.35	0.29	83

of variation is 0.7%. For replicate samples of Suttocide®A at the 10 ppm level, the average assay was found to be 9.34 ppm $\pm$ 0.27 ppm at the 95% confidence level. The coefficient of variation is 1.2%. These experiments confirm both the stability of the electrode response as well as the reproducibility of the analysis scheme.

A blind study was conducted for the determination of the Suttocide®A content of four typical cosmetic formulations representing

a broad range of preservative levels and matrix components. The results of the analyses are shown in Table II. As is evident, excellent recovery is obtained for the shampoo, conditioner, and styling gel formulations while acceptable recovery is obtained for the moisturizing facial treatment. It should be noted that the latter is an oil-in-water emulsion which is somewhat more difficult to sample reproducibly compared to the water soluble systems. However, for all cases, the recovery is acceptable for routine testing.

In conclusion, single column anion chromatography with integrated pulsed amperometric detection has been successfully used for the determination of Suttocide®A in cosmetic formulations. The method is relatively simple and fast, with characteristically high specificity and precision even in the presence of complex matrix components.

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