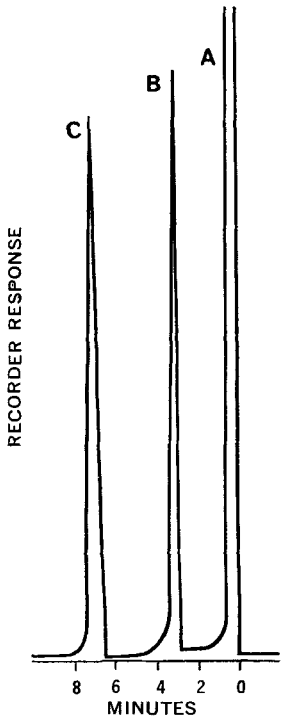

Figure 1—*Gas chromatogram showing relative retention times of methamphetamine and* n-*tridecane. Key: A, solvent; B, methamphetamine; and C,* n-*tridecane.*

simplifies analysis. It was found that unless the methamphetamine hydrochloride is converted to its free base, chromatographic results are spurious and unpredictable.

Both recovery of a known amount of methamphetamine hydrochloride and precision were excellent. Addition of measured amounts of drug to a placebo, with subsequent analysis, yielded 100.3% recovery. Relative standard deviations of numerous analyses were about ±1%.

Investigations are being conducted on the application of this approach to the analysis of other drugs in similar matrixes.

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Determination of Lactate in Parenterals Containing Reducing Sugars

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Abstract □ Reducing sugars in parenterals containing sodium lactate interfere with the determination of the lactate. An acid–diatomaceous earth column separation of sodium lactate (as lactic acid) is proposed which uses ether as the eluting solvent. After esterification, the lactate is converted to a hydroxamic acid which reacts with iron(III) to form a reddish-purple complex which absorbs at 515 nm. The standard deviation of the method is 1.07% of the average amount declared as being present. The method is rapid and can be used for parenterals containing 20% or less of reducing sugar. Parenterals containing partially hydrolyzed protein give high results.

Keyphrases □ Lactate determination—parenterals containing reducing sugars □ Hydroxamic acid formation—lactate analysis □ Column chromatography—separation □ Colorimetric analysis—spectrophotometer

The charring and titration method for the determination of sodium lactate given in the USP (1) is satisfactory for preparations listed in that compendium. The official method, however, does not work satisfactorily for the analysis of sodium lactate in parenterals and other nonofficial products that contain reducing sugars, phosphates, stabilizers, and other substances which produce titratable bases or color interferences when charred.

Hillig (2) suggested a continuous ether extraction of lactic acid from an acidified dairy product sample followed by a colorimetric assay. Boisson (3) used a similar extraction procedure for the removal of lactic acid from solutions that also contain dextrose, except the sample solution was saturated with ammonium sulfate prior to extraction. Dalrymple (4) analyzed nonofficial parenterals for sodium lactate by using the Hillig extraction procedure followed by titration of the extracted lactic acid with sodium hydroxide solution. Staruszkiewicz (5) proposed a GLC method for lactic and succinic acids in eggs, in which the acids were extracted into ether by a continuous extraction procedure, esterified, and extracted into chloroform before injection. These methods are time consuming and

suffer from interference by stabilizers, such as sulfites, and by carryover of the inorganic acid.

This paper reports the development of an acid–diatomaceous earth column separation of sodium lactate (as lactic acid) from reducing sugars and other interfering substances, using ether as the eluant. After esterification, the lactate is converted to a hydroxamic acid which reacts with iron(III) to form a reddish-purple complex which is measured photometrically at a wavelength of 515 nm. The color development is a modification of the Goddu *et al.* (6) procedure for organic esters.

EXPERIMENTAL

Apparatus—A chromatographic tube, Pyrex, 2.20 × 25 cm., constricted at one end to 0.4 × 5.0 cm., was used. An aluminum tamping tool was used to fit the chromatographic column. All photometric measurements were made on a Cary model 15 or model 11 spectrophotometer.

Reagents and Solutions—All reagents were ACS, USP, or NF grade. The following were used:

1. Diatomaceous earth[1], acid-washed grade.
2. Ethyl ether, neutral—Agitate 400 ml. of ethyl ether in a separator with 25.0 ml. of 1.0 *N* NaOH and separate. Wash ether with 25-ml. portions of water by agitation and separation until the wash water is neutral to litmus.
3. Sodium hydroxide (12.5% w/v methanolic)—Reflux 12.5 g. of NaOH pellets with 100 ml. of methanol for several minutes and cool.
4. Sodium hydroxide (0.1% w/v methanolic)—Add 1 ml. of 12.5% methanolic NaOH to 124 ml. of methanol.
5. Hydrochloric acid (5% v/v methanolic)—Add 5 ml. of HCl (37% w/w) to 95 ml. of methanol.
6. Hydroxylamine hydrochloride (12.5% w/v methanolic)—Dissolve 12.5 g. of $NH_2OH \cdot HCl$ in 100 ml. of methanol. Adjust, using phenolphthalein, so that 5.0 ml. requires 2.5 ± 0.2 ml. of 12.5% methanolic NaOH.
7. Ferric perchlorate stock solution—Add 10.0 ml. of perchloric acid (70% w/w) to 0.800 g. of standard iron wire in a 50-ml. beaker. Cover beaker with a watch glass and place on a steam bath until the iron dissolves, approximately 5 min. (CAUTION: the iron dissolves with almost explosive violence if the solution is heated too rapidly.) Cool to room temperature and dilute to 100 ml. with absolute ethanol.
8. Ferric perchlorate analytical solution—Dilute 10.0 ml. of stock ferric perchlorate and 3.0 ml. of perchloric acid (70% w/w) to 250 ml. with absolute ethanol. Discard after 3 days.
9. Calcium lactate standard solution (0.5 mg./ml.)—Dry hydrous calcium lactate to constant weight at 120°. Dissolve 100.0 mg. of anhydrous standard in 10 ml. of water and dilute to 200 ml. with alcohol USP. This standard is equivalent to 0.413 mg./ml. lactic acid.

PROPOSED METHOD

Preparation of Chromatographic Column—Place a loose pad of glass wool in the bottom of a chromatographic column; add approximately 30 g. of anhydrous, granular sodium sulfate; and cover with a loose pad of glass wool. Thoroughly mix 0.5 ml. of ammonium sulfate (50% w/v) with 1.0 g. of diatomaceous earth and transfer to the column. Pack evenly with the tamping rod. Prepare the sample and transfer to the column as directed under *Preparation of Sample*.

Preparation of Sample—Dilute an appropriate aliquot of sample with alcohol USP to give a sodium lactate concentration of between 0.25 and 0.45 mg./ml. Pipet a 10.00-ml. aliquot into a 100-ml. beaker and evaporate to dryness on a steam bath. Cool residue to room temperature and dissolve in 2 ml. of 1 *N* HCl. Mix thoroughly with 3.0 g. of diatomaceous earth and transfer to the column in two portions, packing each evenly. Dry wash the beaker with 0.5 g. of diatomaceous earth and transfer to column. Cover with a loose pad of glass wool.

[1] Celite 545, Johns-Manville Corp., New York, N. Y.

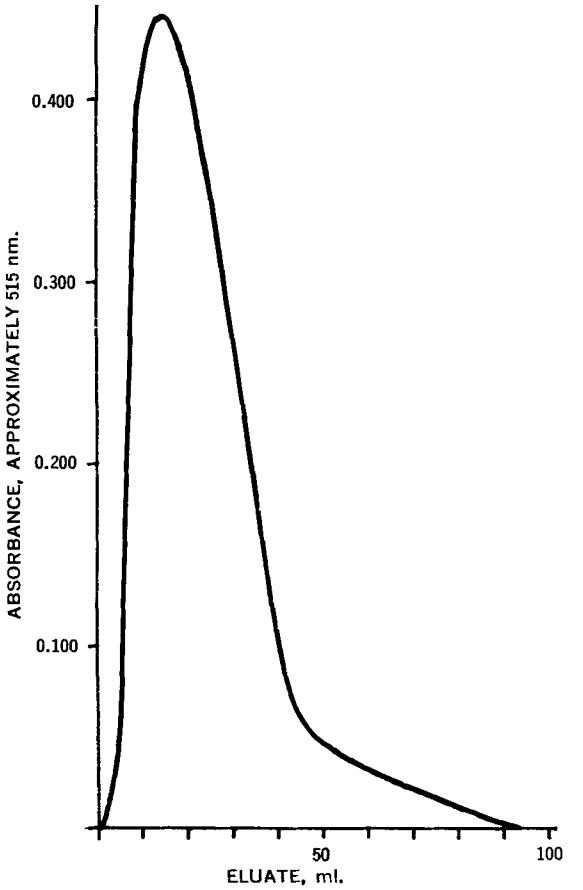

Figure 1—*Typical lactic acid elution curve.*

Preparation of Standards—Pipet 0-, 2.0-, 4.0-, 6.0-, and 8.0-ml. aliquots of the standard calcium lactate into separate 125-ml. glass-stoppered conical flasks. These aliquots are equivalent to 0 (blank), 0.83, 1.65, 2.48, and 3.30 mg. of lactic acid, respectively. To each, add 1.0 ml. of 0.1% methanolic NaOH, and evaporate to dryness on the steam bath. Continue as directed under *Color Development* beginning with "add 1.0 ml. of 5% methanolic HCl. . .." In certain of the recovery studies, the standards were treated as samples and adsorbed on diatomaceous earth for column separation. For this

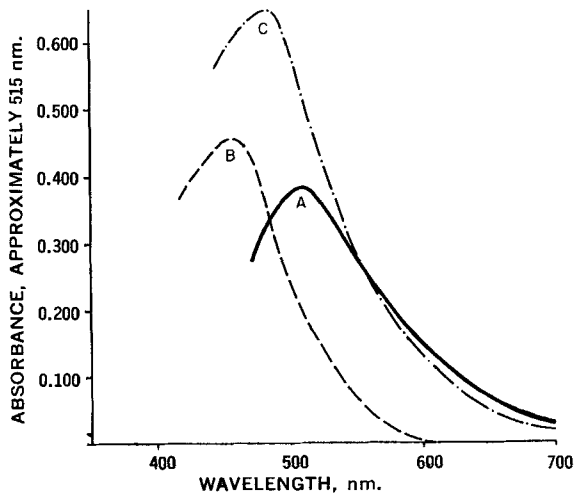

Figure 2—*Dextrose interference in absorbance measurements. Key: A, sodium lactate, 1.5 mg.; B, dextrose, 100 mg.; and C, sodium lactate, 1.5 mg. plus 100 mg. of dextrose.*

Table I—Sodium Lactate in Commercial Preparations

Product	Sodium Lactate Declared, mg./100 ml.	Found mg./100 ml.	Found Percent of Declared
5% Dextrose with electrolyte No. 75[a]	220	230.6 228.2	104.8 103.7
5% Invert sugar with electrolyte No. 2[a]	280	307.0 308.6	109.6 110.2
Sodium lactate injection USP	187	182.5 180.8	97.8 96.9
5% Dextrose with electrolyte No. 48[a]	260	273.7 267.1	105.3 102.7
10% Dextrose[b] with electrolyte No. 48	260	250.4	96.3
20% Dextrose[b] with electrolyte No. 48	260	257.1	98.9

[a] These electrolyte solutions contain various amounts of $NaC_3H_5O_3$ NaCl, KCl, $MgCl_2$, K_2HPO_4, NaH_2PO_4, and $NaHSO_3$. [b] Prepared from the commercial sample containing 5% dextrose in electrolyte No. 48 by addition of dextrose.

procedure, pipet the aliquots into 100-ml. beakers and continue as directed under *Preparation of Sample*, beginning with "evaporate to dryness on a steam bath. . .."

The color is stable for at least 1 hr. and follows Beer's law in the range of 0.5–4.0 mg. of lactic acid per 50 ml. Even though a previously prepared calibration curve can be used satisfactorily in many cases, a series of standards should be run with each set of samples to guard against unexpected variations in temperature affecting the results.

Chromatographic Separation—Use a 125-ml. glass-stoppered conical flask as the column eluate receiver. Rinse the sample beaker with two 10-ml. portions of neutral ether and add to the column. Follow with neutral ether as needed to maintain a flow rate of approximately 6 ml./min. After 100–110 ml. of the ether eluate has been collected, rinse the tip of the tube with neutral ether and remove the flask. Add 2.0 ml. of 0.1% methanolic NaOH to the flask and evaporate to dryness on a steam bath.

Color Development—(Keep the flasks stoppered during the color development procedure except for addition of reagents.) To each individual flask, add 1.0 ml. of 5% methanolic HCl and swirl to hasten solution. After the sample has dissolved, add 1.5 ml. of 12.5% methanolic hydroxylamine reagent, swirl, and allow to stand for 20 min. Add 1.5 ml. of 12.5% methanolic NaOH, mix, and allow to stand for 10 min. Add 25.0 ml. of the ferric perchlorate analytical solution, and agitate thoroughly to ensure complete solution of any precipitated ferric hydroxide. Allow to stand 10 min. Filter the solution quantitatively through dry Whatman 41H paper into a 50-ml. volumetric flask, using alcohol USP to rinse the flask and paper. Dilute to volume with alcohol USP. Measure the absorbance at 515 nm. against the blank. If a recording spectrophotometer is used, record the visible spectrum from 490 to 700 nm.

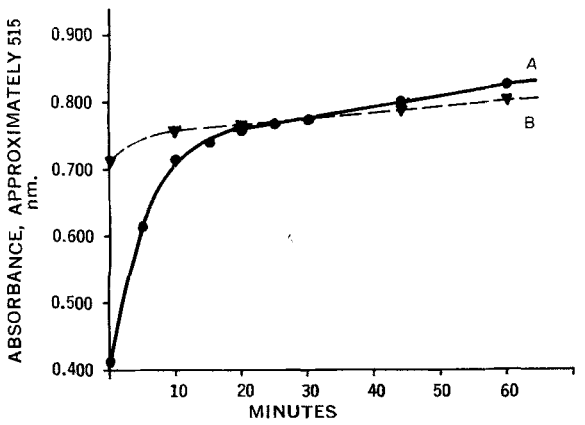

Figure 3—*Effect of time in alkaline and acidic media on net absorbance of 2.1 mg. of sodium lactate. Key: A, acidic solution at room temperature (25°); and B, alkaline solution at room temperature (25°).*

METHOD DEVELOPMENT

Chromatographic Separation—In the proposed procedure, the sodium lactate in the sample is converted to lactic acid by dissolving in 1 *N* HCl before adsorption on diatomaceous earth for transfer to the column. As a consequence, the chromatographic separation involves the selective removal of lactic acid from the column by partition between the ether eluant and the hydrochloric acid solution adsorbed on the diatomaceous earth. Elution studies indicate that hydrochloric acid concentrations up to 6 *N* apparently have no effect upon the elution pattern, but concentrations between 3 and 6 *N* often cause colored blanks, probably due to the leaching of iron from the diatomaceous earth even though the acid-washed grade is used. The proposed method utilizes 1 *N* HCl, which does not cause colored blanks and which gives a satisfactory elution pattern. With this concentration, 99.4% of a 6.6-mg. sample of lactic acid is removed from the column in the first 50 ml. of ether eluant; therefore the 100–110 ml. specified in the method ensures complete removal of even larger lactic acid samples than are recommended. A typical elution pattern for 3.90 mg. of lactic acid is shown in Fig. 1.

Interference Studies—Diethyldithiocarbamate, which is often used as a preservative for ether, interferes with the color development step. It reacts with the color reagent to form a magenta-colored compound which absorbs in the same region as the compound produced with lactic acid (7). The ether is washed with sodium hydroxide to remove the diethyldithiocarbamate and then with water to remove the excess base.

Early results indicated the loss of lactic acid during ether evaporation. Two milliliters of 0.1% methanolic NaOH is sufficient to convert the lactic acid to sodium lactate and does not interfere with the esterification step. Larger volumes tend to cause erratic results in the absorbance developed, probably due to partial neutralization of the methanolic HCl used in the esterification step.

Water interferes with both the esterification and color development by causing a decrease in absorbance of approximately 3%/0.05 ml. of water. Consequently, it is essential that the eluate be evaporated to complete dryness, even though the sodium sulfate trap used in the column is effective in the removal of water.

Dextrose and other reducing sugars interfere with the color development step and must be eliminated by the chromatographic separation. Figure 2 shows the amount of interference in the color development step caused by 100 mg. of dextrose on the absorbance of a 1.5-mg. sample of sodium lactate. In many parenterals the ratio of sugar to sodium lactate is approximately 20 to 1, so the column must be capable of removing at least 100 mg. of dextrose for the sample sizes recommended in the proposed procedure.

Early studies indicated that some dextrose was eluted from the column with the ether eluate when the column was prepared using the acidic sample layer only. A 50% ammonium sulfate solution adsorbed on diatomaceous earth was instituted as a trap layer below the sample layer, and it improved the results significantly. However, replicate studies indicated occasional high values which could not be explained satisfactorily except that the ether was evidently removing small amounts of water and dextrose from the aqueous traps. Incorporation of a layer of anhydrous sodium sulfate below the ammonium sulfate trap effectively removes any water and dextrose. Certain organic acids, such as acetic, succinic, citric, tartaric, and ascorbic acids, are partially or completely eluted from the column with the lactic acid and, consequently, interfere with the procedure by producing iron(III) hydroxamate chelates which absorb in the same region. Since none of the known parenterals containing sodium lactate includes other organic salts in the formulation, these acids need not be considered unless their presence is stated in the declared formulation of the parenteral.

One sample which contained partially hydrolyzed casein in addition to dextrose, sodium lactate, and other electrolytes gave suspiciously high results when run by the recommended procedure. The interference was believed to be due to amino acids or other decomposition products which were being eluted with the lactic acid during the chromatographic step. Efforts to eliminate or offset the interference were unsuccessful, even though the interference could be reduced by precipitation of the unhydrolyzed casein before evaporation of the diluted original sample. An average value

of 130% of the declared amount was obtained by the recommended procedure. This value was reduced to 115% of the declared amount by precipitation of the unhydrolyzed casein before analysis. Thus, samples that contain hydrolyzed protein cannot be run satisfactorily by the recommended procedure.

Esterification and Hydroxamic Acid Formation—The reaction in the acid medium is assumed to be mainly esterification and that in the basic medium mainly hydroxamate formation (8). Most esterification procedures for methylation of lactic acid call for a dehydrating agent or heat during the esterification (9). Various dehydrating agents including gaseous hydrogen chloride, concentrated sulfuric acid, and anhydrous calcium chloride (10) were investigated. The reaction was also carried out in methylene chloride and ethylene chloride to eliminate the necessity of a dehydrating agent. Satisfactory yields of methyl lactate were obtained with each procedure. However, the small amount of lactic acid which must be esterified in the proposed procedure suggested that the reaction might be quantitative in the presence of the large excess of hydroxylamine without the presence of a dehydrating agent or anhydrous solvent, since the reaction of the hydroxylamine with the ester should help drive the esterification reaction to completion. Time studies on the esterification and hydroxamate formation reactions were instituted.

The amount of time allowed in the acid medium before addition of the methanolic base was varied while the amount of time between addition of the methanolic base and the iron color-producing reagent was kept constant. The amount of time allowed between the addition of the methanolic base and the iron color-producing reagent was then varied while the time in the acid medium was kept constant. The results are shown in Fig. 3. Both reactions increase rapidly at first and then reach a plateau of slow increase after a given length of time. The optimum times were found to be 20 min. in the acid medium and 10 min. in the basic medium.

The actual absorbing species measured is the iron(III) chelate of the hydroxamic acid formed by the reaction of methyl lactate and hydroxylamine (6). The reaction is quantitative at room temperature and should not be heated since the absorbance of the chelate is affected greatly by temperature. After the color is developed, raising the temperature to approximately 60° causes rapid loss in absorbance and a change in the absorption maximum. If the solution is then cooled to room temperature, the original color returns but the absorbance is permanently decreased by varying amounts, depending upon the length of time at the elevated temperature. At room temperature, the color is stable for approximately 60 min. but then fades slowly. After 3 hr. the absorbance is reduced to approximately 94%.

The presence of even small amounts of water affect the stability of the color developed. For example, the use of 95% ethanol USP instead of absolute ethanol in the preparation of the iron(III) perchlorate solutions causes the fade rate to increase to approximately 14%/hr.

RESULTS AND DISCUSSION

The results of the analyses of representative commercially available parenterals which contain sodium lactate and reducing sugars are shown in Table I. The standard deviation, as calculated from the difference in duplicates (11), is 2.62 mg./100 ml., 1.07% of the average amount declared as present. The results are satisfactory in the presence of as much as 20% dextrose, as shown in the last sample in the table.

The standard deviation for six replicate determinations on a sample containing 5% dextrose was 0.054 mg., which represented 1.90% of the average amount found. Recovery of 4.10 mg. of standard by the procedure averaged 4.09 mg., with a standard deviation calculated from the range (12) of 0.053 mg. or 1.29%.

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