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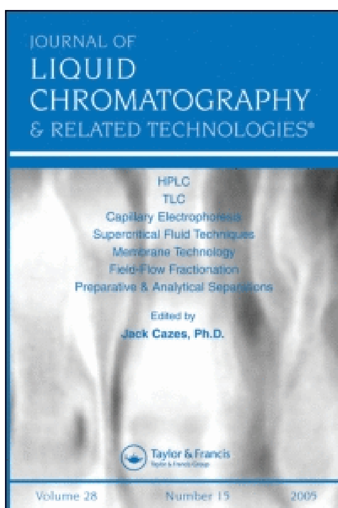

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EVALUATION OF AN ION EXCLUSION/DIRECT CONDUCTIVITY METHOD FOR QUANTITATING ACETIC AND LACTIC ACIDS IN PHARMA-CEUTICAL LVP BASE FORMULATIONS

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EVALUATION OF AN ION EXCLUSION/DIRECT CONDUCTIVITY METHOD FOR QUANTITATING ACETIC AND LACTIC ACIDS IN PHARMACEUTICAL LVP BASE FORMULATIONS

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ABSTRACT

Organic acids (e.g., acetic, lactic and gluconic acid) are common components in Large Volume Parenteral (LVP) base solutions. The characterization of such solution products is necessary for production control and product release. A chromatographic method, which couples separation by anion exclusion with direct conductivity detection, has been developed for this purpose. The evaluation of this method, carried out in a manner consistent with United States Pharmacopeia (USP) and International Council for Harmonization (ICH) guidelines, is summarized in this manuscript. The method meets the acceptance

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criteria for in-process and/or product release testing with respect to accuracy, linearity, range, precision, and specificity.

INTRODUCTION

Organic acids (e.g., acetic, lactic and gluconic acid) are common components in Large Volume Parenteral (LVP) base solutions. Baxter Healthcare is a major vendor in this business and has a product line which consists of 14 solution formulations. The characterization of such solution products is necessary for production control and product release. In the highly regulated pharmaceutical industry, analytical methods, which would be used in such applications, must demonstrate the necessary performance characteristics for parameters such as accuracy, precision, linearity, and range. Additionally, these methods must possess a high degree of specificity in sample matrices which may include electrolytes, sugars, and their associated decomposition products and impurities. In a practical sense, the methods must be sufficiently rugged and robust that they could be implemented effectively on a routine basis. The process of demonstrating that an analytical method possesses performance characteristics which meet established quality criteria is termed validation. Guidelines for conducting validation studies have been published by national and international regulating bodies (1,2).

Various ion chromatographic methods have been proposed for the quantitation of organic acids in a wide variety of sample matrices. Such methods include ion exchange (3–5,13) and ion exclusion (6–17) separations. Supporting detection methods included suppressed (4,5,7–11) and non-suppressed (3,6,14) conductivity, potentiometry, (12) indirect photometry, (13) and low wavelength direct UV (14–17). Given the analytical requirements of the application studied herein, ion exclusion chromatography with non-suppressed conductivity detection was judged to be the most viable option.

The ability of Ion Chromatography (IC) with direct conductivity to perform the required testing was evaluated. The purpose of this anuscript is to establish the performance specifications for the methodology. This study specifically focused on two organic acids, acetic acid and lactic acid.

EXPERIMENTAL

Test Samples

Given the number of LVP base formulations, which contain organic acids, it is not practical to validate an analytical method in all such formulations. An

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alternate approach is to define a subset of these formulations, which includes members whose compositions either encompass or approximate the population extremes. LVP base formulations can be categorized into two groups, depending on whether they contain sugar (dextrose) or not. While the LVP base formulations possess a wide range of compositions, they generally are similar in terms of the major components and differ in terms of proportions. Thus, it is possible to identify specific codes whose composition could be considered to be representative of a number of these formulations. Such representative matrix codes were examined in this study. The benefits of using the matrix codes are practical in nature and primarily revolve around issues of efficiency. Matrix codes used in this study are summarized in Table 1.

The actual test samples used in this study were prepared from commercially available raw materials and/or existing pharmaceutical products. The LVP products are steam sterilized and such treatment can potentially result in formulation decomposition products (especially in dextrose-containing formulations), which may have an impact on specificity. Thus, all test articles were prepared from steam sterilized solutions.

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Analytical Method

The analytical method used in this study coupled an ion exclusion separation with direct conductivity detection. The operational parameters used in this study are outlined in Table 2. The assay was implemented using a Waters 510 pump, Waters 431 conductivity detector, an electronically actuated sample value, a Micromertics 728 autosampler, and a Hewlett Packard 4910 ChemServer software package for data collection and analysis.

Design of the Evaluation Experiments

The specific design of each of the evaluation experiments is discussed in each evaluation parameter section in the Results and Discussion portion of this manuscript.

RESULTS AND DISCUSSION

General Method Development

Initial acetate assessments were performed using water as the standard matrix. Such a strategy produced sample recovery results which were

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Table 1. LVP Base Formulations Used in This Study

Formulation	Composition [g/L for Sugar (Dextrose) or Molar for Ions]										Ionic	mOsmol/L	pH
	Sugar (2)	Na	K	Ca	Mg	Cl	PO4	Ac (1)	Glu (1)	Lac (1)	Strength	(calc)	Range
Used in the Assessment for Acetate and Lactate													
Plasmalyte R	—	0.14	0.01	0.0025	0.0015	0.103	—	0.047	—	0.008	0.162	312	4.0–6.5
5% Dextrose/Plasmalyte M	50	0.04	0.016	0.0025	0.0015	0.04	—	0.012	—	0.012	0.073	377	4.0–6.0
Used in the Assessment for Acetate only													
Plasmalyte A pH 7.4	—	0.14	0.005	—	0.0015	0.098	—	0.027	0.023	—	0.149	294	6.5–8.0
5% Dextrose/Plasmalyte 148	50	0.14	0.005	—	0.0015	0.098	—	0.027	0.023	—	0.149	547	4.0–6.0
Used in the Assessment for Lactate only													
5% Dextrose/LR/KCl (40)	50	0.13	0.044	0.0015	—	0.149	—	—	—	0.028	0.151	605	4.0–6.5
5% Dextrose/Electrolyte 75	50	0.04	0.035	—	—	0.048	0.03	—	—	0.02	0.076	402	4.0–6.5

Note: (1) Ac = acetate, Glu = gluconate, Lac = lactate. (2) Sugar = dextrose, hydrous.

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Table 2. Analytical Conditions for the Evaluated Ion Chromatographic Method

Parameter	Operating Value
Column	Alltech Anion Exclusion Column, 300 × 7.8 mm (or equivalent)
Mobile Phase	2 mM Sulfuric Acid
Mobile Phase Flow Rate	0.7 mL/min
Column and Detector Temperature	40°C
Detector	Conductivity (non-suppressed)
Injection Size	100 μL for acetate, 20 μL for lactate
Sample Preparation	Appropriate dilution (e.g., 1/10) with water

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reproducibly low, in the range of 95–98%. Investigation of this effect indicated that the sample's salt matrix was responsible for this effect, although the exact mechanism was not clear. However, since the effect was observed for acetate but not lactate, it is believed that it is related to the lower pK_a and higher volatility of acetate. While the formulations, which contain acetate have various proportions of chloride salts and would be diluted for analysis by different factors, the total salt content in the diluted samples (expressed as chloride) falls within a narrow range (0.005–0.010 M). Thus, one solution to the "salt effect" would be to prepare standards in a matrix that had a similar salt content. This salt concentration was 470 mg/L sodium chloride. While other mechanisms exist for dealing with the "salt effect", standard matrix matching is the most practically viable. While these other methods, which include standard additions and sample pre-treatment with silver-containing exchange media, would effectively deal with the salt effect, they are not as time and cost efficient as simple matrix matching. Thus, the assessment was performed using matrix matched (with respect to total chloride loading) standards.

The general assay conditions developed for acetate were also examined for lactate. While lactate was well resolved for both acetate and the formulations components, its mass response (peak height) was much larger than that of acetate. There are at least two reasons for this. Firstly, lactate elutes earlier, producing a sharper peak. Secondly, lactate (the actual analyte detected) is a larger mass fraction of its salt (sodium lactate) than is acetate (sodium acetate trihydrate). Thus, a sample, which is equal mass concentration in sodium lactate and sodium acetate trihydrate, will have a larger lactate response.

This difference in lactate versus acetate response resulted in a situation were lactate peaks overloaded the detector when a 300 mg/L dilution target and 100 μL was used. Thus, the lactate target was changed to 100 mg/L (as sodium lactate) and two sample injection volumes were examined (5 μL and 20 μL). Use

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of the smaller sample size produced inadequate precision and thus, the 20 μL injection size was examined in this study.

The optimized analytical method was characterized with respect to its performance specifications. Performance specifications were established for the following performance parameters: linearity, accuracy, precision, specificity, and range. Additional performance parameters (e.g., ruggedness and robustness) were also examined. The specific evaluation experiments performed and the results of those experiments are summarized as follows.

Linearity

Design

300 mg/L sodium acetate was determined to be the appropriate sample preparation target. This target level was achieved by appropriate sample dilution. The standard range assessed was 50 to 150% of the optimum target. Specifically, linearity was assessed across this range with five standard solutions; prepared at 50% (150 mg/L), 75% (225 mg/L), 100% (300 mg/L), 125% (375 mg/L), and 150% (450 mg/L) of the optimum target. Each standard was injected in triplicate in a random order. Performance specifications generated included r^2, % y-intercept (of the 100% value), precision, and accuracy (% recovery). The standards were prepared in such a way that they contained approximately 470 mg/L sodium chloride.

The target analyte concentration used in this study was 100 mg/L sodium lactate. This target was achieved in the formulation samples by appropriate sample dilution. The standard range assessed was 50 to 150% of the optimum target. Specifically, linearity was assessed across this range with five standard solutions; prepared at 50% (50 mg/L), 75% (75 mg/L), 100% (100 mg/L), 125% (125 mg/L), and 150% (150 mg/L) of the optimum target.

Results

The results of this assessment are shown in Table 3. The results indicate that an effective, accurate, and precise linear correlation can be made between analyte response (peak area) and acetate concentration over the range of 150 to 450 mg/L as sodium acetate trihydrate (50 to 150% of the sample dilution target, 300 mg/L). Similar behavior is obtained for sodium lactate over the range of 50 to 150 mg/L as sodium lactate. The resulting calibration curve for sodium acetate is illustrated in Figure 1.

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Table 3. Summary of Results, Linearity Assessment (1)

Parameter or Property	Result, Acetate	Result, Lactate
Slope:	2.86853×10^{-7}	$1.97134 \times 10^{\times 7}$
Intercept:	−1.768 mg/L	−1.985 mg/L
% y-intercept (2):	−0.59%	−1.95%
r^2	0.9998	0.9997
% Recovery, 50% Level:	100.2%	98.32%
% Recovery, 75% Level:	99.99%	100.6%
% Recovery, 100% Level:	100.1%	100.9%
% Recovery, 125% Level:	99.70%	100.3%
% Recovery, 150% Level:	100.2%	99.44%
Precision (%RSD), 50% Level	0.22	0.35
Precision (%RSD), 75% Level	0.75	0.49
Precision (%RSD), 100% Level	0.61	1.00
Precision (%RSD), 125% Level	0.78	0.57
Precision (% RSD), 150% Level	0.48	0.40

Notes: (1) Calibration model is concentration = slope (peak area) + intercept. (2) % y-intercept is relative to mean response at the 100% level.

Accuracy

Design

Formulation blanks were prepared to contain all the formulation components at their prescribed formulation level except for the analyte of interest. Such blanks were prepared in glass containers and subjected to steam sterilization. In order to assess accuracy, portions of the blanks were fortified so that they contained 80, 100, or 120% of the amount of the specification level of sodium acetate trihydrate or sodium lactate. The spiking was accomplished as part of the sample dilution process.

The appropriately diluted samples were injected once (blank) or three to five times (spiked). Accuracy was assessed as the % recovery of the spike, which was calculated as follows:

$$\% \text{ recovery} = (C_{\text{measured}}/C_{\text{calculated}}) \times 100\%$$

where, C_{measured} is the concentration measured in the spiked sample and $C_{\text{calculated}}$ is the calculated amount of spike added, based on the preparation of the spiked sample.

Additionally, sample linearity was assessed by constructing a "calibration curve" of sample spike level versus sample response. Performance specifications

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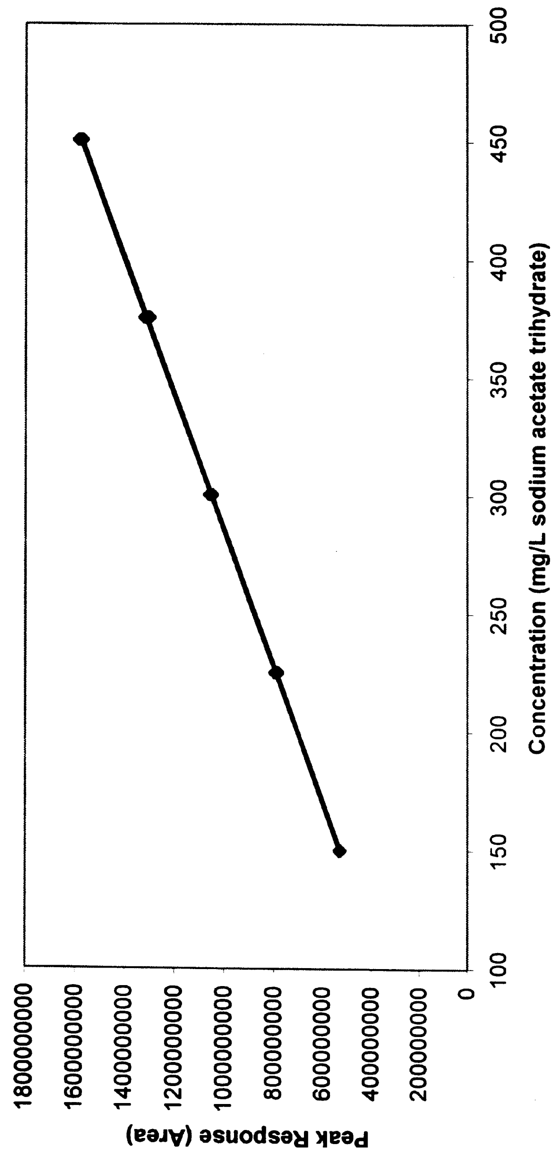

Figure 1. Standard calibration curve, acetate response versus standard concentration. Each of the five standards was injected three times in random order.

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generated included r^2, % y-intercept (of the 100% value), and accuracy (% Recovery).

Results

Accuracy results using the salt matched standards are summarized in Tables 4 (A and B) and 5. A typical plot of sample concentration versus chromatographic response for sodium acetate trihydrate is shown in Figure 2. Recoveries generally fall in the range of 98–102% and the "calibration curve" is characterized by a high degree of linearity.

Precision

Design

Portions of the 100% formulation spike were assayed in replicate (5 times). The replicate analyses included both dilution as required and injection. Precision was expressed as the % relative standard deviation of the replicate analyses.

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Results

The precision results are summarized in Table 6 and are typically less than 1.5%.

Specificity

Design

The chromatograms of the formulation blank and spiked formulation (80%) were examined, specifically in the elution region of the target analyte. The specific concern was whether the formulation blank contains a peak in the analyte elution region of the spiked sample. A peak was deemed to be present if a baseline disturbance greater than $3 \times S/N$ (signal to noise) was observed.

A second specificity assessment was performed by preparing a test sample cocktail which contained 13 potential dextrose impurities, related substances, "foreign sugars", and/or decomposition products (for example, references 18–20). These compounds were chosen based on chemical considerations. Compounds present in the cocktail included: D-cellobiose, formic acid, D-fructose, 2-furoic acid, B-gentobiose, D-gluconic acid lactone, D-gluconic acid, 5-(hydroxymethyl) furfural (5-HMF), isomaltose, DL-lactic acid, levulinic acid,

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Table 4A. Summary of Results and Accuracy Assessment for Acetate

		Concentration (mg/L, 1)		
Formulation	Spike Level	Target	Mean Result	% Recovery
I (2)	80	5409	5379	99.5
	100	6611	6677	101.0
	120	7813	7867	100.7
II (3)	80	1502	1501	100.0
	100	1802	1801	100.0
	120	2102	2095	99.6
III (4)	80	3004	3004	100.0
	100	3862	3858	99.9
	120	4720	4713	99.9
IV (5)	80	3005	2982	99.2
	100	3606	3573	99.1
	120	4507	4465	99.1

Notes: (1) Concentrations are in mg/L as sodium acetate trihydrate. (2) Plasmalyte R, sample dilution factor = 20. (3) Plasmalyte M and 5% Dextrose, sample dilution factor = 10. (4) Plasmalyte A, pH 7.4, sample dilution factor = 14.28 (7 to 100). (5) Plasmalyte 148 and 5% Dextrose, sample dilution factor = 10.

Table 4B. Summary of Results and Accuracy Assessment for Lactate

		Concentration (mg/L, 1)		
Formulation	Spike Level	Target	Mean Result	% Recovery
A (2)	80	669.77	681.24	101.7
	100	837.21	839.07	100.2
	120	1004.7	998.11	99.34
B (3)	80	1163.2	1195.2	102.8
	100	1453.9	1489.2	102.4
	120	1744.7	1775.4	101.8
C (4)	80	2374.8	2391.6	100.7
	100	3053.3	3022.0	98.97
	120	3731.8	3767.3	101.0
D (5)	80	1832.0	1807.5	98.66
	100	2210.2	2190.8	99.12
	120	2646.2	2592.1	97.95

Notes: (1) Concentrations are in mg/L as sodium lactate. (2) Plasmalyte R, sample dilution factor = 50/6 = 8.3333. (3) Plasmalyte M and 5% Dextrose, sample dilution factor = 100/7 = 14.2857. (4) 5% Dextrose/LR/KCl(40), sample dilution factor = 100/3 = 33.333. (5) 5% Dextrose and Electrolyte 75, sample dilution factor = 20.

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Table 5. Curve Fit Parameters, Accuracy Model (1)

Formulation	Curve Fit Parameters			
	Slope	Intercept (3)	% y-Intercept (2)	Correlation (r^2)
Acetate				
I (4)	5.53886×10^{-6}	191.3	2.89	0.9993
II (5)	2.90032×10^{-6}	−29.272	−1.62	0.9997
III (6)	4.10919×10^{-6}	−25.857	−0.67	0.9994
IV (7)	2.90102×10^{-6}	−13.188	−0.37	0.9998
Lactate				
A (8)	1.67531×10^{-6}	−34.860	−4.16	0.9996
B (9)	2.77026×10^{-6}	−58.986	−4.06	0.9989
C (10)	6.28152×10^{-6}	43.331	1.42	0.9986
D (11)	3.92439×10^{-6}	7.889	0.36	0.9990

Notes: (1) Model is concentration = slope (peak area) + intercept. (2) % y-intercept relative to the 100% formulation level. (3) In units of mg/L as sodium lactate. (4) Plasmalyte R. (5) Plasmalyte M and 5% Dextrose. (6) 5% Dextrose/LR/KCl(40). (7) 5% Dextrose and Electrolyte 75. (8) Plasmalyte R. (9) Plasmalyte M and 5% Dextrose, diluted by 10. (10) Plasmalyte A, pH 7.4, diluted by 14.29 (7 to 100). (11) Plasmalyte 148 and 5% Dextrose.

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maltose, and D-mannose. The cocktail was prepared so that each of these compounds was individually present at a level, which was the molar equivalent of 5% of the amount of dextrose present in products typically assayed by the IC method (e.g., 5% by weight dextrose hydrous). The cocktail was diluted 1 to 10 to mimic the typical dilution, which would be used to analyze a dextrose-containing product. Such a diluted sample was prepared unspiked and spiked to contain approximately 300 mg/L sodium acetate trihydrate. Additionally, a standard was prepared which also contained 300 mg/L sodium acetate trihydrate but in a water matrix. These solutions were repetitively injected into the chromatographic system using the operational parameters for the acetate determination. Similar test preparations were made for sodium lactate, however, this sample contained only twelve of the compounds (DL-lactic acid not used for the obvious reason that lactate was the analyte of interest). Furthermore, the spike level for the sodium lactate was 100 mg/L.

Results

See Figure 3 for a typical chromatogram. Given the composition of the formulations (high salt content) and the detection mechanism (conductivity), it is

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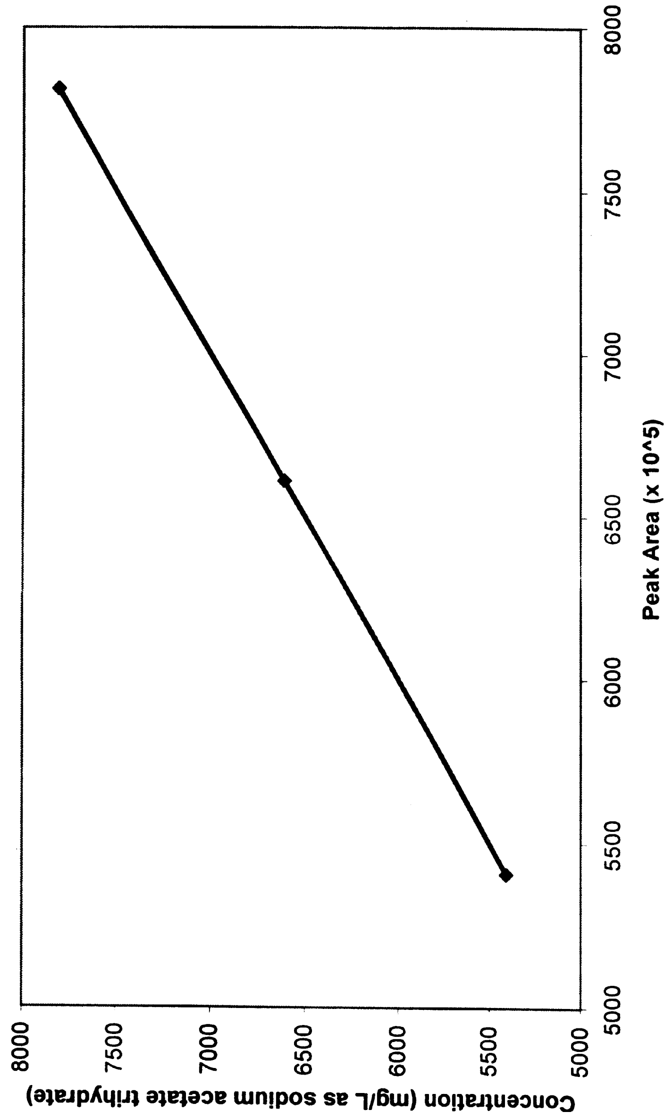

Figure 2. Sample calibration curve, acetate response versus concentration in the formulation I (Plamalyte R) matrix. Each sample was injected a minimum of three times.

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Table 6. Summary of Results, Precision Assessment

Formulation	Target Level	Dilution	Precision (%RSD, 3)
Acetate			
I, Plasmalyte R	6605 (1)	20	0.35
II, Plasmalyte M & 5% Dextrose	1651	5	0.64
III, Plasmalyte A, pH 7.4	3603	10	0.14
IV, Plasmalyte 148 & 5% Dextrose	3603	10	1.24
Lactate			
A, Plasmalyte R	837.2 (2)	8.333	0.53
B, Plasmalyte M & 5% Dextrose	1453	14.2857	0.75
C, 5% Dextrose/LR/KCl(40)	3053	33.333	0.70
D, 5% Dextrose & Electrolyte 75	2210	20	0.57

Notes: (1) mg/L as sodium acetate trihydrate. (2) mg/L as sodium lactate. (3) %RSD = % relative standard deviation, 5 preparations/injections.

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not surprising that the sample chromatograms all contain a large void volume response. While in some formulations the lactate and acetate peaks elute on the tail of the void response, this does not interfere with the effective integration of the acetate peak.

Additionally, it is observed that none of the chromatograms of the formulation blanks contained a peak in the elution region of acetate.

Finally, it is noted that none of the 13 compounds and/or chemical entities present in the test cocktail eluted in the elution region of acetate. The acetate peak in the spiked test cocktail eluted cleanly with no evidence of interring responses. Similarly, it is noted that none of the 12 compounds and/or chemical entities present in the test cocktail eluted in the elution region of lactate. The lactate peak in the spiked test cocktail eluted cleanly with no evidence of interring responses. Thus, method specificity in a dextrose matrix was confirmed for both analytes. It is observed in passing, that the test solutions analyzed in this study contained no discernable responses at the elution times of the compounds and/or chemical entities present in the test cocktail (other than lactate).

Sensitivity

Sensitivity is not a relevant validation parameter for the intended application of these assays. However, LOQ was determined (as $10 \times S/N$) as 9.9 mg/L for sodium acetate trihydrate and 3.6 mg/L for sodium lactate.

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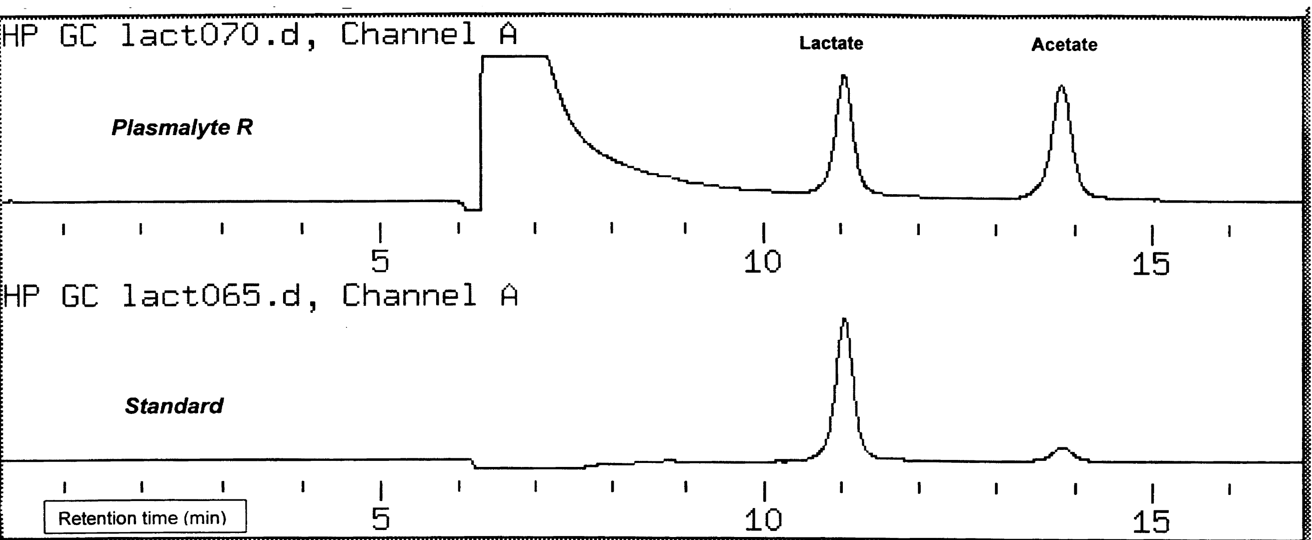

Figure 3. Typical chromatograms, formulation A (Plasmalyte R, 80% level) and a standard (water matrix) containing approximately 100 mg/L each, of the sodium salts of lactate and acetate (trihydrate). While the sample contains matrix components that produce a large void response, the lactate and acetate peaks are well resolved and elute cleanly with no specificity issues. The lactate assay conditions were used (20 µL sample, 8.333 dilution).

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Robustness

Design

Operational variables, which may influence the performance of the assay include mobile phase concentration and column/detector temperature. Thus, a similar experiment was performed using the following experimental variations: Mobile phase: 1, 2 or 3 mM sulfuric acid; Column/Detector Temperature: 35, 40, 45°C. The experiment was performed as follows: For each operational condition, the 100% standard was injected three times. Additionally, the formulation blank and 100% spiked formulation for Plasmalyte R and 5% Dextrose/Plasmalyte M were injected once for sodium acetate trihydrate. Similarly, the formulation blank and 100% spiked formulations for Plasmalyte R and 5% Dextrose/Electrolyte 75 were injected once for sodium lactate.

The impact of these changing conditions on performance was assessed by (1) examination of the blank injections for potentially interfering peaks, (2) precision of the three injections of the standard, and (3) accuracy was expressed as the ratio of the responses obtained for the spiked samples versus the standard.

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Results

The results of the robustness assessment are summarized in Table 7. The operational changes made in mobile phase composition and operating temperature had little relative impact on performance. Assay performance with respect to specificity, precision, and accuracy was not impacted by the changing operational parameters. While changing system temperature changed the absolute magnitude of the detector background, such changes had little if any impact on the magnitude of the analyte response. Alternatively, changing the mobile phase composition affected both the magnitude of the baseline conductivity and the magnitude of the analyte peak. As the concentration of sulfuric acid in the mobile phase decreased, the background conductance decreased and the analyte response increased. In fact, an increase in signal of approximately 2.7 times was obtained when the mobile phase composition was changed from 3 mM to 1 mM sulfuric acid.

Under typical circumstances, the increase in analyte response observed in going from 3 mM to 1 mM sulfuric acid would be a desirable circumstance. However, with the detector used in this study, such an increase in sensitivity resulted in acetate peaks, which overloaded the detector. To compensate this effect, the acetate experiments with the 1 mM sulfuric acid mobile phase were performed with a smaller injection size (50 µL versus 100 µL). To avoid this for lactate, the robustness assessment was performed with a 5 µL sample size. While

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Table 7. Robustness Assessment

Performance Characteristic		Result				
		Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
Acetate Method						
Property	Parameter	35°C, 2 mM	45°C, 2 mM	40°C, 2 mM	40°C, 1 mM	40°C, 3 mM
Specificity	Peaks? (2)	No	No	No	No	No
Precision	% RSD (3)	1.41	0.55	0.52	0.20	0.80
Accuracy	% Recovery (5)	98.9	98.8	98.5	99.5	101.9
	% Recovery (5)	95.7	93.6	95.7	97.5	96.6
Sensitivity	Response (7)	3562133	3239804	3358812.1	2305902	6119903
Lactate Method						
Specificity	Peaks? (2)	No	No	No	No	No
Precision	% RSD (3,4)	0.14	1.04	6.34	1.94	2.55
Accuracy	% Recovery (8)	91.1	97.9	97.4	96.3	97.5
	% Recovery (9)	97.6	99.4	104	96.2	99.5
Sensitivity	Response (10)	6659234	6512191	6239683	4676965	11706450

Notes: (1) These are the nominal operating conditions. (2) Specificity was assessed by examining chromatograms of the blank formulations for interfering peaks. (3) % RSD = % relative standard deviation of three injections of STD100. (4) In the case of the other conditions, these were 3 sequential injections. In the case of condition 3, these were from three random injections. (5) At the 100% spike level in the Plasmalyte R formulation. (6) At the 100% spike level in the 5% Dextrose and Plasmalyte M formulation. (7) Response factor = (concentration of sodium acetate trihydrate in STD100, mg/L)/(peak area, STD100). (8) At the 100% spike level in the Plasmalyte R formulation. (9) At the 100% spike level in the 5% Dextrose/Electrolyte 75 formulation. (10) Response factor = (concentration of sodium lactate in STD100, mg/L)/(peak area, STD100).

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use of sub-optimal assay conditions produced data of poorer quality than is cited elsewhere, the data is of sufficient quality to allow for a comparison of performance across operating conditions.

While all the data did not meet the acceptance criteria established previously for each individual validation parameter (for example, accuracy), this was expected due to the necessary use of non-optimal conditions (injection volume). The goal of detecting changes in performance, which is the intent of a robustness assessment, could still be achieved with the experimental design employed. Since performance changes between the various operating conditions used were small, it was concluded that the assay was robust with respect to small changes in operating temperature and mobile phase concentration.

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Ruggedness

Design

The accuracy experiment performed on a single product code (5% Dextrose/Plasmalyte 148, formulation IV for acetate, 5% Dextrose/Electrolyte 75, matrix D, for lactate) was performed by a second analyst using a second column and a second preparation of analytical reagents (standards and mobile phase). The second analyst used the same formulation blank prepared by analyst 1. Ruggedness was evaluated via a comparison of the recovery results obtained by the two analysts. In the case of the acetate method, the ruggedness experiment was performed using water as the standard matrix.

Results

The results of the ruggedness assessment are summarized in Table 8. While all the acetate data did not meet the acceptance criteria established previously for accuracy, this was expected due to the use of water standards. The goal of detecting changes in performance, which is the intent of a ruggedness assessment, could still be achieved with the experimental design employed. Since the results obtained by both analysts were similar, the assays were judged to be rugged.

Response Stability

The experiments were performed in such a manner that one of the chromatographic runs included nearly 80 injections and endured for nearly 24 hours. Such an extended run allows for the assessment of response stability

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Table 8. Ruggedness Results

Parameter	Property	Result, Analyst I	Result, Analyst II
Acetate			
Accuracy (2)	% Recovery, 80%	94.39	96.90
	% Recovery, 100%	95.07	97.00
	% Recovery, 120%	96.15	96.50
Precision	% RSD, 80%, n = 3	0.26	0.42
	% RSD, 100%, n = 5	1.24	0.45
	% RSD, 120%, n = 3	1.05	0.37
Calibration Curve (1)	Slope	3.07618×10^{-6}	2.80886×10^{-6}
	Intercept	44.08	2.52
	% y-intercept	1.22	0.07
	Correlation coefficient	0.9987	0.9990
Lactate			
Accuracy	% Recovery, 80%	98.66	96.40
	% Recovery, 100%	99.12	98.30
	% Recovery, 120%	97.95	97.01
Precision	% RSD, 80%, n = 3	0.75	0.78
	% RSD, 100%, n = 5	0.57	0.58
	% RSD, 120%, n = 3	0.51	0.37
Calibration Curve (1)	Slope	3.92349×10^{-6}	3.85344×10^{-6}
	Intercept	7.888	28.00
	% y-intercept	0.36	1.27
	Correlation coefficient	0.9990	0.9983

Note: (1) Calibration curve is a plot of sample preparation concentration versus measured response. (2) Obtained using water as the standard matrix. It is expected that such a standard matrix would produce low absolute values for accuracy. However, changes in the measured accuracy between Analyst I and Analyst II can be used to assess method ruggedness.

since standards at the 75%, 100%, and 125% levels were injected throughout the course of the run.

Response stability was assessed in two ways. In the first approach, the % relative standard deviation (%RSD) of all injections was calculated. In the second approach, the % change in response between the first standard injection (r1) and the last standard injection (r2) was calculated as:

$$\%\text{Change} = \{(r1 - r2)/[(r1 + r2)/2]\} \times 100\%$$

The results of the response stability calculations are shown in Table 9. Given the extended period of operation, the response changed very little over time.

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Table 9. Response Stability

Standard Level, % of Target	% Relative Standardization	% Response Change
Acetate (1)		
75	0.91	−1.57
100	1.60	−1.26
125	0.74	−0.41
Lactate (2)		
75	1.73	0.08
100	1.17	0.37
125	0.66	−0.29

Notes: (1) 7 injections of each standard made over the course of a single analytical run that lasted nearly 24 hours. (2) 5 injections of each standard made over the course of a single analytical run that lasted nearly 20 hours.

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CONCLUSION

HPLC methods, based on ion exclusion separation and conductivity detection, have been developed and evaluated for the analysis of LVP base solutions for their acetate and lactate content. The methods, as described in Table 2, produce data that meets performance expectations for characteristics including linearity, accuracy, precision, specificity, and response stability. Ruggedness and robustness were demonstrated, as the measured performance characteristics were not materially impacted by changes in operating conditions (mobile phase concentration and operating temperature) and operating systems. Utilization of the evaluated method would require its formal validation.

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