Development and Validation of an Ion-Exclusion Chromatographic Method for the Quantitation of Organic Acids in Complex Pharmaceutical Products

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Abstract

Effective quantitation of organic acids (acetic, malic, and lactic acids) present in total parenteral nutrition (TPN) pharmaceutical products for final product release purposes requires accuracy, precision, and specificity but less than optimal sensitivity. A chromatographic method relying on ion-exclusion separation and low-wavelength ultraviolet detection has been developed and validated to quantitate these organic acids in matrices containing 10-fold or greater excesses of other constituents (sugars, amino acids, and other inorganic salts) with no sample preparation other than dilution. Mobile phases at slightly different pH values effectively eliminates matrix-related interferences that were observed during the analysis of several products. The validation procedure used is discussed in terms of its strategy and results. Ultimately, the assay is found to be appropriate for the release testing of several compositionally diverse TPN products.

Introduction

Organic acids in pharmaceutical products act as buffers, stabilizers, and appropriately soluble counterions for active ingredients. Partial or total parenteral nutrition (TPN) infusion products are complicated solutions that contain organic acids, amino acids, sugars, vitamins, and inorganic salts and are used to cover energy and electrolyte requirements, particularly in a variety of insufficiency situations. As compounded products, their composition is confirmed prior to final product release. Because the results of such testing dictates the product release decision, the assays that are used must be accurate and precise. The complicated nature of these solutions, which may contain more than 50 g/L of total dissolved solids, also places a premium on the analytical method's ability to differentiate between the organic acid analytes and the product matrix. However, because the concentration of the organic acids in these types of products is typically large (1 g/L or greater), high analytical sensitivity is not required and in fact may be counterproductive, requiring large sample dilutions and small material weighings.

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 (1–4) and ion-exclusion (5–14) separations with supporting detection methods including suppressed (2,3,6–10) and non-suppressed (1,5,12) conductivity, potentiometry (11), indirect photometry (4), and low-wavelength direct ultraviolet (UV) radiation (12–14). Given the analytical requirements of the application studied herein, ion-exclusion chromatography with direct low-UV detection was judged to be the most viable option. In this research, the performance of the developed chromatographic method is described within the context of its validation for use in release testing of TPN products. The validation strategy employed and its results are discussed, and the need to modify mobile phase pH to eliminate matrix-specific analytical interferences is presented.

Experimental

Chromatographic system and conditions

Separations were accomplished with a Bio-Rad (Hercules, CA) Aminex HPX-87H ion-exclusion column (300 × 7.8 mm) coupled to a Micro-Guard Cation H+ guard column. The two mobile phases used were 0.15% sulfuric acid and 0.005% phosphoric acid. The operating conditions were the same for both mobile phases. The mobile phase flow rate was 0.8 mL/min, the detection wavelength was 210 nm, the injection size was 20 µL, and the columns were temperature-controlled at 60°C.

The chromatographic system employed was modular in its composition and included an ABI (Ramsey, NJ) Spectroflow 400 pump, an Alcott (Norcross, GA) model 728 autosampler, an Alcott model 235 electronically actuated injection valve, an ABI model 757 UV detector, an appropriate strip chart recorder, and a computer data acquisition system.

Test articles

The test articles were 14 product codes representing TPN solutions manufactured by P.T. Pfrimmer Infusol Indonesia (Bekasi, Indonesia). These product codes were obtained after their distribution but were within their individual expiration dates. The general chemical composition of the product codes that were tested is summarized in Table I. Products were prepared for anal-

ysis by dilution with water; the dilution factors that were used ranged from no dilution to a factor of 10 (1 mL product to 10 mL final volume).

Validation strategy

The validation strategy that was utilized is summarized in Table II and detailed in the following discussion.

Preliminary screening

To assess method applicability (analytical accuracy and the nature of the chromatographic profile for each product code), a preliminary screening study was performed. Based on the product composition and the assay operating range, a sample preparation strategy was defined for each product prior to the screening experiments. Products were prepared and analyzed according to this defined procedure versus standards at levels bracketing the anticipated operating range. The analysis run time was twice as long as required to elute the analyte. In this way, late-eluting peaks were observed that dictated the total run time. For the validation process to continue, the level of the analyte found in the product must agree with the label claim or manufacturing specifications to within ± 10%, and the analyst must observe no detector response that might interfere with the accurate quantitation of the analyte.

Accuracy and precision

Accuracy and precision were evaluated using products fortified with a known additional amount of analyte. This approach was adopted rather than comparing analytical results to label claims because the manufacturing tolerances are such that the label claim only approximates the product's analyte concentration, especially after significant storage in the field (due to degradation). The test article matrix was prepared by diluting the product to a level twice as dilute as would normally be required for its analysis, resulting in a test article matrix that contained 50% of the sample matrix and 50% of the nominal level of the analyte. The analysis test samples were prepared by fortifying the test article matrix with the analyte of interest to levels of approximately 80, 100, and 120% of the product's nominal analyte level. The fortification was accomplished by adding a small amount of a concentrated stock solution into the test article matrix.

Test samples fortified at the 80 and 120% levels were analyzed with duplicate injections and those fortified at the 100% level with five injections made within a single analytical run for each product code. Precision was calculated as the relative standard deviation (RSD) of the five replicate injections of the 100% test sample. Accuracy was calculated as the ratio of the fortified concentration found in the sample versus the calculated fortification level. The fortified concentration found in the sample was the difference between the analyte level found in the fortified sample and the analyte concentration found in the unfortified test article matrix. Accuracy was expressed as a percentage (the accuracy ratio multiplied by 100%). The acceptance criteria were that the precision must be better than 2% (RSD < 2%) and the accuracy at each fortification level must be 100% ± 4. These acceptance criteria are consistent with recommendations made for product release assays whose acceptance range is 90–110% of the label claim (15).

Linearity and range

Linearity was addressed by preparing standards at three levels encompassing the target sample preparation concentration range. Standards were injected in triplicate in a random order into the chromatograph. A linear regression analysis of the analyte concentration versus peak response data was performed. The standard concentration range that was evaluated was approxi-

Table I. Approximate Compositions of the Product Codes of Interest in this Study

	Approximate level of component in the product (g/L)					
Product code	Amino acids*	Sugars†	Acetate‡	Malate§	Lactate**	Other††
A			3.40	0.67		40 (D), 7.7 (I)
B		50 (SO)	5.17			5.9 (I)
C	25	70 (SO), 55 (X)	3.40 (#)			5.9 (I)
D		150 (M)	2.04			2.6 (I)
E	35	30 (SO), 30 (X)	2.82 (#)			5.5 (I)
F	68	50 (X)	1.83	7.00		3.5 (I)
G	25	25 (SO), 25 (X)	1.36 (#)	3.00		3.2 (I), 1.0 (V)
H	36	50 (SO), 50 (X)	1.36 (#)	2.01		3.2 (I), 1.0 (V)
I	50		0.68 (#)			3.3 (I)
J	5.0	50 (SO)	1.36	2.15		2.9 (I), 1.0 (V)
K	92		7.46 (#)			
L	54	25 (S), 25 (X)		3.76 (#)		2.4 (I)
M	26	50 (X)		7.00		4.4 (I)
N					2.24	60 (S), 7.5 (I)

* Total of all amino acids present in the product code.
† X = xylitol, SO = sorbitol, and M = mannitol.
‡ As sodium acetate trihydrate; products denoted with a (#) did not contain this salt but contained this ion, contributed by a different compound.
§ As L-malic acid; products denoted by a (#) did not contain this compound but contained malate from another compound.
**As L-lactic acid.
†† I = inorganic salts, S = hydroxylethyl starch, D = Dextran 40, and V = vitamins and related substances.

mately 80% of the lowest anticipated product level to 120% of the highest product level, which is consistent with recommendations for check specification assays with product ranges of 90–110% of the label claim (16).

To demonstrate acceptable linearity, the correlation coefficient (r^2) from the linear regression analysis had to be greater than 0.999, and the ratio of the intercept versus the mean response of the lowest concentration standard had to be less than 0.10.

Ruggedness

Ruggedness testing was focused on setup and column-to-column variation. The assay was set up on two different chromatographic systems using two different analytical columns (three different setups total), and each system was supplied with independently prepared supporting analytical reagents (mobile phase, standards, system suitability samples, etc.). Each product sample was spiked to the 100% analyte level and assayed on each of the three chromatographic systems via five replicate, sequential injections. The resulting data set for each sample (15 determinations) was used to calculate a sample-specific population mean and RSD.

Acceptable ruggedness requires that the measured population mean for each sample and fortified sample agree to within 10% (i.e., the percentage of the population mean versus the label-claimed concentration had to fall in the range of 90–110%). Additionally, method reproducibility was assessed via the RSD and required that the population RSD be no greater than 5% (RSD ≤ 5%). The precision requirement is consistent with the

Table II. Validation Parameter, Test Methods, and Acceptance Criteria

Validation parameter	Test method	Acceptance criteria
Assay suitability (preliminary screening)	Linearity: Assess range of sample concentrations and potential instrumental performance to establish target calibration range and appropriate sample dilution scheme.	Sample dilution scheme to be in place before formal validation begins. Calibration range to encompass entire anticipated analyte concentration range ± 20% of the lowest and highest anticipated concentration.
	Accuracy: Test of appropriately diluted product.	Measured level to be within 10% of label claim
	Specificity: Examination of sample chromatogram allowed to develop two times peak of interest elution time.	Analyte peak elutes cleanly with no integration interferences; proper run time established.
Linearity	Three random injections of three calibration standards over the range defined above.	Correlation coefficient (r^2) ≥ 0.999. Intercept: ≤ 10% of low concentration standard response.
Accuracy	Method of spiking: Product sample is diluted twice as much as defined above. Diluted matrix is spiked to contain 80, 100, and 120% of the analyte present in a normally diluted sample. Spiked and unspiked sample portions are analyzed (minimum of duplicate injections each).	Recovery of anticipated spike must be 100% ± 4.
Precision (within run)	Sample spiked at 100% level is injected five replicate times within an analytical run.	RSD of five replicate injections ≤ 2.0%.
Ruggedness	Accuracy (between runs): Sample spiked at 100% level is analyzed on three different analytical setups.*	Mean recovery of the 100% spike must be 100% ± 10.
	Precision (between runs): Five replicate injections of the 100% spiked sample made in each of three analytical setups.*	Population RSD ≤ 5%.

* An analytical setup refers to a specific chromatographic system or column couple operated with freshly prepared analytical reagents.

Table III. Results of Preliminary Screening Experiment: Acetate

	Performance in 0.15% H_2SO_4 mobile phase		Performance in 0.005% H_3PO_4 mobile phase	
Product code	% Recovery versus label	Elution profile*	% Recovery versus label	Elution profile*
A	98.8	C		
B	98.7	C		
C	99.2	C, L		
D	99.5	C		
E	94.6	C, L		
K	101	C		
F	216	C	100	C
G	117	C	94.8	C
H	236	C, L	91.0	C, L
I	241	C, L	87.5	C, T, L
J	116	C, L	98.6	C, L

* C: Acetate peaks eluted cleanly with no directly or indirectly interfering peaks.
L: Large late-eluting peaks resulted in increased total run time.
T: Acetate peak eluted on the tail of a larger detector response.

Table IV. Linearity Results

Analyte	Concentration range (g/L)	Correlation coefficient	Intercept ratio	Typical product dilution
Acetate*	0.3–1.9‡	0.99972	0.047	1:2–1:10
Acetate†	0.3–0.9‡	1.0000	0.088	none–15:50
Malate	0.15–0.75§	1.0000	0.025	none–1:10
Lactate	0.20–0.80**	0.99998	–0.008	1:5

* With 0.05N H_2SO_4 mobile phase.
† With 0.005% H_3PO_4 mobile phase.
‡ As sodium acetate trihydrate.
§ As L-malic acid.
** As L-lactic acid.

Table V. Analytical Accuracy: Acetate Assay

	Accuracy as mean percent recovery of spike expressed as percent of labeled product level			
	80%	100%		120%
Product code		Within run (five replicates)	Between runs (15 replicates)	
In 0.15% H_2SO_4 mobile phase				
A	96.1	98.6	96.6	97.0
B	98.9	99.3	97.8	100.4
C	100.5	103.7	105.5	99.7
D	97.6	98.5	101.2	101.9
E	104.3	103.2	106.1	99.0
K	100.5	103.6	104.9	99.0
In 0.005% H_3PO_4 mobile phase				
F	97.6	97.3	101.9	96.7
G	97.9	97.4	102.4	96.3
H	99.1	99.9	untested	97.6
I	96.7	96.0	99.0	95.5
J	99.1	98.8	100.5	97.8

general observation that a method's reproducibility (precision related to ruggedness) can be expected to be 2 to 3 times greater than its repeatability (precision under normal conditions) (17).

Specificity

Specificity was assessed via visual examination of product chromatograms. The elution region around the analyte peak was examined for interfering peaks using appropriate response and retention time scale enhancements. The acceptance criterion for specificity was visual confirmation of baseline resolution between the analyte peak and all other system responses. Given the varied nature of the products evaluated in this study, a single placebo that mimicked all the product could not be identified. However, individual products were sufficiently similar in major chemical composition but differed in the presence or absence of one or more of the analytes of interest so that some products could be used as placebos for others. In such cases, specificity was demonstrated by the absence of peaks in the analyte elution region in chromatograms generated with the placebo products.

Results and Discussion

Acetate assay

Preliminary screening

The results of the preliminary screening validation experiment are summarized in Table III. The initial mobile phase was 0.15% sulfuric acid, and all products were screened using this assay. Approximate analyte retention times were as follows: 7.0 min for malate, 9.0 min for lactate, and 10.6 min acetate (Figure 1). As shown in Table III, five product codes (F, G, H, I, and K) did not meet the acceptance criteria for accuracy and thus were not compatible with this mobile phase. In each of these products, the acetate peak was significantly broadened versus the peak observed in a standard, suggesting that co-elution between acetate and an interfering ion was occurring.

Attempts to remove the interfering entity by ion-exchange sample preparation were unsuccessful but suggested that the interferant was ionic with a pK_a value somewhat lower than that of acetate. Thus it was felt that the elution characteristics of the interferant could be changed by changing the mobile phase pH, whereas the elution properties of acetate would be unaffected. Producing such a pH change while preserving some mobile phase buffering capacity required that the mobile phase counterion be changed from sulfate to phosphate. In mobile phase scouting experiments, a mobile phase with a pH of approximately 3.3 (0.005% phosphoric acid) was found to produce an acetate response whose magnitude was consistent with the product's labeled acetate levels. This mobile phase was adopted for all products for which the sulfuric acid mobile phase was inappropriate. Although the retention times were not materially changed in the 0.005% phosphoric acid mobile phase, the malate response was significantly decreased. The decrease in response represents the pH difference between the mobile phases (less than 2.0 for the sulfuric acid, approximately 3.3 for the phosphoric acid) and the pK_a value of the analytes. With a pK_a value of 3.4, malic acid was partially ionized in the phosphoric acid mobile phase, and thus a smaller response was observed. Both lactate and acetate have pK_a values that are sufficiently high (3.8 and 4.8, respectively) that their speciation and thus their response were not materially affected by the difference in pH exhibited by the two mobile phases.

To avoid analytical interferences that arise when entities from a previous injection elute during the chromatographic run for a later injection, the analysis time for several product codes had to be increased (e.g., Figure 2). However, in all cases wherein the appropriate mobile phase was used, the acetate peak eluted cleanly

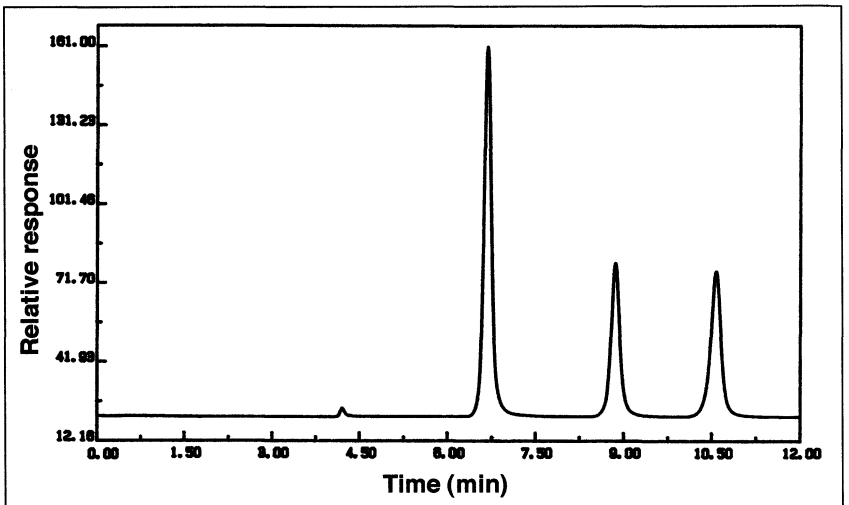

Figure 1. Typical chromatogam of a standard showing the elution order of maleic, lactic, and acetic acids. The separation was performed with a 0.15% sulfuric acid mobile phase at an analyte concentration of approximately 1 g/L of each analyte. Though a similar profile was obtained with the 0.005% phosphoric acid mobile phase in terms of run time, the malic acid response was a factor of 3 less due to the effect of mobile phase pH on analyte speciation.

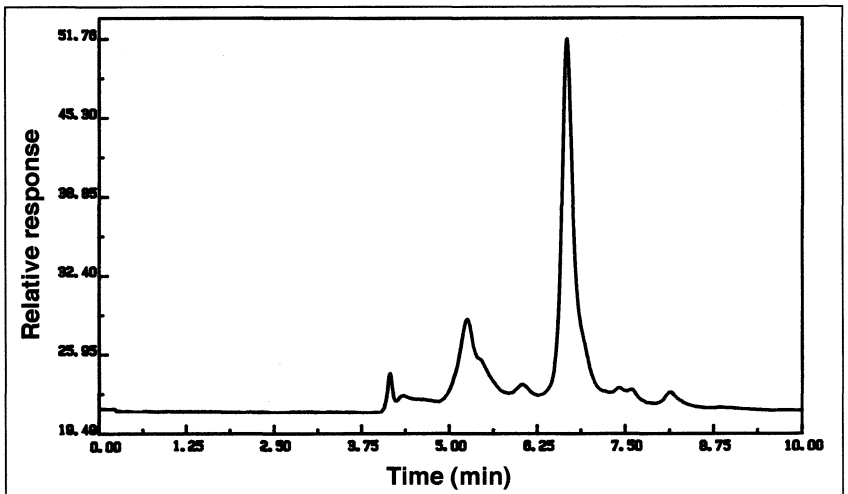

Figure 2. Chromatogram obtained during the analysis of product code G for its L-malic acid content (0.15% sulfuric acid mobile phase). Late-eluting peaks not shown in this chromatogram dictated that the total run time for this code be nearly 20 min.

with no obvious interferences arising from poorly resolved product components.

Linearity and range

The results of the linearity and range assessment for acetate using both mobile phases is shown in Table IV. The ranges were different for the different mobile phases due to the nature of the products for which each mobile phase was appropriate. In either case, the peak area response was linearly related to the analyte concentration over the specified concentration range, and the best fit regression line was characterized by an intercept that was small with respect to the magnitude of the response arising from the lowest concentration standard. Thus calibration bias at the low end of the calibration range was minimal. Product dilution ranging from none to 10 was required to produce working samples whose acetate level fell within the calibration range.

Table VI. Analytical Precision: Acetate Assay

Sample code	Precision (RSD [%]) Within run (five replicates)	Between runs (15 replicates)
In 0.15% H_2SO_4 mobile phase		
A	0.35	0.96
B	0.27	0.70
C	0.17	0.65
D	1.52	1.39
E	0.26	0.98
K	0.72	1.05
In 0.005% H_3PO_4 mobile phase		
F	1.26	3.38
G	0.65	3.71
H	0.82	untested
I	0.75	2.45
J	0.60	3.64

Table VII. Results of Preliminary Screening Experiment: Malate Assay

Product code	% Recovery versus label	Elution comments*
A	96.9	C, L1
F	97.7	C, L1
G	97.8	C, L
H	103.4	C, L
L	103.5	C, L
M	97.3	C, L1

* C: Malate peak eluted cleanly with no directly or indirectly interfering peaks.
L: Large late-eluting peaks resulting in increased total run time.
L1: Later eluting acetate peak resulting in increased total run time.

Accuracy and precision

The accuracy and precision of the acetate method is summarized in Tables V and VI. Intra-run data were related to accuracy and precision, and inter-run data reflected analytical ruggedness. In the case of both accuracy and precision, the assay's performance met the acceptance criteria established for these validation parameters in each product code tested.

Ruggedness

Ruggedness was assessed by analyzing the product samples fortified at the 100% analyte level on three different chromatographic setups. These setups included two guard–analytical column couples with two instrumentally similar chromatographic systems that differed in terms of the exact piece of equipment used. For example, though both systems utilized a Spectroflow 757 detector, the specific detector used in both systems was different. Uniquely prepared analytical solutions (standards, system suitability solutions, and mobile phases) were freshly prepared and used for each setup. The results of the ruggedness assessment for the acetate assay are shown in Tables V and VI. The acceptance criteria for reproducibility and long-term accuracy were met for all product codes.

Specificity

The specificity of the assay could be qualitatively examined in the preliminary screening experiment by close examination of the analyte responses in the product codes. Such examination readily allowed the identification of the analytical issue associated with several of the product codes when the sulfuric acid mobile phase was used. To address specificity in greater detail, two product codes that are formulated without acetate (codes L and M) but are otherwise similar to acetate-containing products were chromatographed. Chromatograms for both product codes contained a response at the elution time of acetate that was similar in nature to the broadened response observed in those products for which the sulfuric acid mobile phase was found to be inappropriate. The fact that both these placebo codes and four of the five product codes for which the sulfuric acid mobile phase was inappropriate contain malate qualitatively suggests that this particular salt may have been responsible for the assay issues observed in the sulfuric acid mobile phase. However, because malate itself eluted cleanly much earlier than acetate, it was not this entity per se that caused the interference.

Table VIII. Analytical Accuracy: Malate Assay

Product code	Accuracy as mean percent recovery of spike expressed as percent of labeled product level: 80%	100% Within run (five replicates)	100% Between runs (15 replicates)	120%
A	99.0	98.9	98.9	98.2
F	98.4	97.9	100.0	97.3
G	96.8	97.9	98.9	99.2
H	102.9	100.4	99.4	100.9
J	98.4	96.0	95.6	94.1
L	99.6	98.8	100.2	99.0
M	98.3	98.3	102.4	98.1

Malate assay

Preliminary screening

The results of the preliminary screening validation experiment are summarized in Table VII. An acceptable analytical performance was obtained for all malate-containing product codes with the 0.15% sulfuric acid mobile phase. Total run time for all product codes was twice the elution time of malate due to the presence of late-eluting peaks in the chromatograms.

Other validation parameters

Linearity of response over the calibration range of 0.15–0.75 g/L as L-malic acid met the validation acceptance criteria (Table IV). Products could reach this calibration by dilutions that ranged from none to 10. The assay met the acceptance criteria for accuracy, precision, and ruggedness (Tables VIII and IX). Assay specificity was demonstrated by close examination of the analyte peaks during the preliminary screening experiments. Additionally, three placebo products (codes B, C, and I) that contain no malate but many of the formulation components of the malate-containing products were chromatographed under the conditions used for the malate assay. No significant interfering peaks were observed in the elution region of malate in the chromatograms of the placebo products.

Lactate assay

Product N contained lactic acid and large amounts of dissolved solids but no other weak acids that might elute under the employed chromatographic conditions. The screening experiment indicated that acceptable performance with the sulfuric acid mobile phase product (0.20–0.8 g/L lactic acid) could be reached by a 1:5 sample dilution. The calibration curve for this range met the acceptance criteria (correlation coefficient of 0.99998, intercept ratio of –0.008). Intra-run accuracy (101.8%) and precision (RSD, 0.24%) over the entire fortification range (80–120%) met the acceptance criteria. The ruggedness acceptance criteria were met; the measured population accuracy and reproducibility were 101.8 and 0.47% (RSD), respectively. In addition to the assay specificity data provided by the preliminary assessment, specificity was checked in this case by the analysis of a placebo generated by reproducing the product formulation using laboratory-grade reagents. Such a placebo, when subjected to typical steam sterilization stress, produced a chromatogram that contained no significant responses in the lactate elution region.

Table IX. Analytical Precision: Malate Assay

Sample code	Precision (RSD [%]) Within run (five replicates)	Between runs (15 replicates)
A	0.62	0.81
F	0.32	2.53
G	1.35	2.70
H	0.27	2.24
J	0.19	1.15
L	0.39	1.27
M	0.57	2.84

Table X. System Suitability Test Results Obtained Throughout this Study

System description: System number	System description: Column number	Value obtained for the system suitability parameter: Precision*	Resolution†	Tailing factor
1	1	1.83	5.22	1.08
1	1	0.61	5.33	1.04
1	1	0.67	5.20	0.94
1	2	1.28	4.72	1.09
1	2	0.66	4.48	1.05
2	2	0.79	4.69	1.00
2	2	0.57	5.57	1.00
2	2	0.58	5.26	1.16
1	1	1.01	4.44	1.00
1	1	0.59	4.71	0.97
1	1	1.25	4.92	1.03
1	2	1.50	5.24	1.04
2	2	0.79	4.18	0.95
Acceptance criteria		≤ 2.0%	≥ 4.0	≤ 1.5

* RSD (%) of five replicate injections with acetate as the analyte.
† Between acetate and bisulfate.

System suitability

During validation, nearly 1000 injections were made on the various chromatographic systems employed. Because these injections were differentiated over time and between several analytical systems, observed performance trends were particularly useful in terms of establishing system suitability criteria. During the evaluations using the sulfuric acid mobile phase, this possibility was anticipated, and a system suitability test mixture containing acetate and bisulfite was injected throughout the course of this investigation. Bisulfite was chosen as the resolution marker due to its relatively close (but later) elution to acetate. Chromatographic performance parameters examined as a result of the injections of the system suitability test mixture included precision (RSD of five replicate injections), peak shape (tailing factor), and resolution. Precision was included as a diagnostic for the sample introduction and detection components of the chromatographic system. Tailing was included as a diagnostic for column poisoning (encouraging multimodal analyte column interactions), and resolution was a specificity control metric adopted because the chromatograms resulting from many products contained potentially interfering peaks.

A summary of the performance data obtained for the injections of the system suitability test mixture throughout this study are shown in Table

X. The performance of the assay was constant over time and between analytical systems, leading to the system suitability acceptance criteria indicated in Table X.

The bisulfite–acetate test solution utilized with the sulfuric acid mobile phase was not applicable with the phosphoric acid mobile phase. This was because bisulfite, with a pK_a value of 1.8, was ionic at the pH of the phosphoric acid mobile phase and was not chromatographically retained. Thus, in the case of the phosphoric acid mobile phase, a system suitability test mixture containing acetate and lactate was developed.

Conclusion

A high-performance liquid chromatographic method based on ion-exclusion separation and low-wavelength UV detection has been validated for use in the product release testing of compositionally complex pharmaceutical solutions (TPN) for their organic acid content. By controlling mobile phase pH, the analyst can eliminate matrix-related responses which interfere with the analytical responses obtained for one of the analytes of interest (acetate).

References

1. B.S. Lord and R.W. Stringham. Liquid chromatographic determination of organic acids used as pharmaceutical counterions. *Anal. Chem.* **68:** 1067–70 (1996).
2. H. Itoh and Y. Shinbori. Simultaneous determinations of inorganic and organic acids by ion chromatography. *Bull. Chem. Soc. Jpn.* **60:** 1327–32 (1987).
3. R.D. Rocklin, R.W. Slingsby, and C.A. Pohl. Separation and detection of carboxylic acids by ion chromatography. *J. Liq. Chromatogr.* **9:** 757–75 (1986).
4. B.P. Downey and D.R. Jenke. Determination of oxalate in pharmaceutical matrices by indirect photometric chromatography. *J. Chromatogr. Sci.* **25:** 519–24 (1987).
5. J.S. Fritz. Principles and applications of ion exclusion chromatography. *J. Chromatogr.* **546:** 111–18 (1991).
6. K. Fischer, C. Corsten, P. Leidmann, D. Bieniek, and A. Kettrup. Determination of short-chain aliphatic acids in silage effluents by ion exclusion chromatography. *Chromatographia* **38:** 43–51 (1994).
7. H.F. Botte and L. Joergensen. Evaluation of low-conductance eluents for suppressed ion exclusion chromatography. *J. Chromatogr.* **602:** 27–31 (1992).
8. C.A. Accorsi and G. Blo. Determination of volatile and non-volatile organic acids in technical sugar solutions by ion exclusion chromatography. *J. Chromatogr.* **555:** 65–71 (1991).
9. S.R. Bachman and M.E. Peden. Determination of organic acid anions in precipitation by ion chromatography. *Water Air Soil Pollut.* **33:** 191–98 (1987).
10. M.J. Lesh, E.L. Wilkinson, M.R. Zolfaghari, and M.A. Schreiber. High performance liquid chromatographic analysis of lactic and lactic acid lactate in amrinone lactate formulations. *J. Liq. Chromatogr.* **16:** 2415–22 (1993).
11. B.L. De Backer and L.J. Nagels. Potentiometric detection of organic acids in ion exclusion chromatography using different types of liquid-membrane electrodes. *Anal. Chim. Acta* **290:** 259–67 (1994).
12. M. Ye, K. Hill, and R. Walkup. Determination of carboxylic acids by ion exclusion chromatography with non-suppressed conductivity and optical detectors. *Chromatographia* **35:** 139–41 (1993).
13. D.W. Togami, L.G. Treat-Clemons, and D.J. Hometchko. Separation of organic acids in food products. *Int. Lab.* **20:** 29,30,32,33 (1990).
14. D. Grosjean, A. Van Neste, and S.S. Parmer. Analysis of atmospheric carboxylic acids using single column ion exclusion chromatography with ultra-violet detection. *J. Liq. Chromatogr.* **12:** 3007–17 (1989).
15. E. Debesis, J.P. Boehlert, T.E. Givand, and J.C. Sheridan. Submitting HPLC methods to the compendia and regulatory agencies. *Pharm. Technol.* **6:** 120–37 (1982).
16. J.C. Wahlich and G.P. Carr. A practical approach to method validation in pharmaceutical analysis. *J. Pharm. Biomed. Anal.* **8:** 619–23 (1990).
17. H.B.S. Conacher. Validation of methods used in crisis situations: Task force report. *JAOAC* **73:** 332–34 (1990).

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