Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1039 (2004) 147–153
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Validated ion-exclusion chromatographic method for citrate and acetate in medical fluids

S. Karmarkar*, M. Koberda, J. Momani, D. Kotecki, R. Garber

Baxter Healthcare Corporation, Technology Park, Route 120 and Wilson Road, Round Lake, IL 60073, USA

Available online 18 December 2003

Abstract

In this paper we describe the development and validation of a solid-phase extraction procedure, followed by ion-exclusion chromatographic determination of citrate and acetate in medical fluids. The medical fluids contained trace levels of non-polar compounds, which were not of interest for the purposes of assay requirements, but due to their strong affinity towards the ion-exclusion chromatography column necessitated a 180-min long runtime to elute. The developed SPE procedure, based on trapping the hydrophobic compounds, on a reversed-phase material, while allowing analytes of interest elute off unretained, shortened the runtime to 35 min. The procedure is simple since it has only two steps, conditioning of the SPE cartridge with acetonitrile and treating the sample. The SPE procedure followed by ion-exclusion chromatographic determination was successfully validated per the International Conference on Harmonization (ICH) guidelines in terms of specificity, accuracy as recovery versus untreated sample, precision, range, linearity of response, ruggedness, stability of treated samples, and robustness. The validation data showed that the method is specific, accurate, precise, rugged, and robust. The validated method has been routinely used in the manufacturing environment.

Keywords: Validation; Citrate; Acetate

1. Introduction

Ion-exclusion chromatographic separation has been commonly used for the determinations of aliphatic organic acids in a variety of matrices as well as non-ionic analytes of significant pharmaceutical interest including alcohols and carbohydrates. In ion-exclusion chromatography, separation is accomplished using dilute mineral acids as mobile phase, to maintain organic acids in their undissociated forms, and separated ions are detected using suppressed or non-suppressed conductometric or direct UV detection [1]. Limitations on the determination of organic acids in complex matrices have been reported in the literature. Weiss [1] cautions that ion-exclusion chromatography should not be used for the determination of aromatic acids since these acids, due to the π–π interactions with the aromatic rings of the stationary phase polymer, are strongly retained onto the ion-exclusion chromatography (IEC) column. Solid-phase extraction (SPE) cleanup on an ion-exchange cartridge, to selectively retain organic acids while eliminating interfering matrix components, followed by ion-exclusion chromatographic determination has been reported for the determination of organic acids in honey [2]. Schiller et al. [3] employed a polystyrene–divinylbenzene cartridge, for the selective removal of hydrophobic compounds, in series with anion exchange cartridge, for trapping the organic anions in pharmaceutical herbal dry extract. The trapped organic anions, citrate and malate, were then eluted off using 0.1 M TFA for the ion-exclusion chromatographic determination using evaporative light scattering detection. Schneider et al. [4] used an SPE cartridge packed with C_{18} reversed-phase material for the removal of matrix components in red wine followed by ion-exclusion chromatographic determination of organic acids. Prior to ion chromatographic determination of inorganic and organic anions in industrial streams, SPE cleanup was used for the removal of organic matrices [5]. On-line coupling of SPE sample processing with HPLC has also been reported in the literature [6].

In the present study, we initially employed ion-exclusion chromatographic determination of citrate and acetate in medical fluids samples without any sample pre-treatment. The samples contained trace levels of non-polar compounds, which were not of interest for the purposes of

* Corresponding author.
E-mail address: shreekant_karmarkar@baxter.com (S. Karmarkar).

0021-9673/$ – see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/j.chroma.2003.11.057

assay requirements, but due to their strong affinity towards the ion-exclusion chromatography column necessitated a 180-min long runtime to elute (Fig. 2). These late eluting peaks carried over to the chromatograms of multiple injections that followed, when the runtime was set to around 20 min, and affected quantitation of citrate and acetate. A runtime of 180 min, although would resolve the problem, is not acceptable for routine use and various approaches were considered to shorten the runtime to no more than 40 min. First approach considered would have employed reversed phase separation using a column with polar functionality. The second approach would have used a short column, mounted on a switching valve, to trap late eluting compounds while allowing citrate and acetate to elute un-retained. Both of these approaches would have required major changes to the existing validated procedure. The SPE approach, on the other hand, would have changed only sample preparation step of the existing ion-exclusion chromatography method. The SPE approach was then further investigated.

Typically the SPE procedure is employed to selectively eliminate the unwanted matrix components but retain, and often to concentrate, the analytes of interest, which are then eluted off from the SPE device using a suitable solvent [3,7,8]. This multi-step approach seems rational when analytes of interest are present in trace amounts compared with matrix compounds. Since the interfering compounds in the formulation samples under consideration were present in trace amounts (Fig. 2), the multi-step SPE approach would have complicated the cleanup procedure for routine use. On the other hand, trapping the unwanted components on the reversed-phase SPE cartridge and letting the analytes of interest elute off unretained, was deemed simpler.

As ion-exclusion chromatography is a liquid chromatographic method, guidelines for the validation of a LC method in the pharmaceutical industry are readily applicable for validating the ion-exclusion chromatography method [9–12]. In this paper, the ion-exclusion chromatographic method with SPE cleanup was validated per the International Conference on Harmonization (ICH) guidelines in terms of specificity, accuracy as recovery versus untreated sample, precision, range, linearity, ruggedness of SPE treatment, stability of treated samples, and robustness.

2. Experimental

2.1. Solid-phase extraction device and procedure

While optimizing SPE procedure, the following cartridges were evaluated: Maxiclean cartridges packed with 600 mg of C_{18} material (Alltech, Deerfield, IL, USA), Maxiclean cartridges packed with 500 mg of C_{18} Prevail material (also from Alltech), C_{18} cartridge packed with 300 mg of C_{18} material (Waters, Milford, MA, USA), and tC_{18} cartridge, also from Waters, packed with 300 mg of tC_{18} material. The SPE procedure was optimized using Waters tC_{18} cartridge. A disposable, latex-free syringe with luer connection, 10-ml capacity (Becton-Dickinson, Franklin Lakes, NJ, USA), was used for conditioning of the SPE cartridge and loading of sample. In the optimized SPE procedure, the cartridge was conditioned by pushing 10 ml of acetonitrile through it. Using the graduation marks on the syringe, a 9-ml sample was then drawn in another syringe, of which 8-ml was pushed through the cartridge and discarded. The remaining 1-ml sample in the syringe was then pushed through the cartridge and collected in the autosampler vial for the ion-exclusion chromatographic determination.

2.2. Reagents and standards

Mobile phase and standards were prepared using ACS-grade reagents. After preparation, the mobile phase was filtered through 0.22 μm filter. Sodium citrate dihydrate was dried at 180 °C for 18 h prior to use. Mixed calibration standards consisted of 0.6–2.4 g sodium acetate/l and 0.4–1.6 g sodium citrate/l. The resolution solution contained 5 mg sodium tartrate/l and 800 mg sodium citrate/l.

2.3. HPLC system and ion-exclusion chromatography procedure

The HPLC system was Agilent's 1100 system consisting of high-pressure pump with on-line degasser, column oven, variable wavelength detector, and ChemServer Chromatographic Data System. The optimized ion-exclusion chromatography procedure consisted of 25 mM H_2SO_4 as mobile phase at 0.8 ml/min, 20 μl injection, 60 °C column temperature, and UV detection at 210 nm. Separation was performed on Bio-Rad's Aminex micro-guard cation H^+ cartridge (4.6 mm × 30 mm) and HPX-87H analytical column (7.8 mm × 300 mm, Bio-Rad Labs., Richmond, CA, USA). To ascertain removal of any undesired components from the column, prior to starting a run of test solutions and samples, the column was flushed with a solution consisting of acetonitrile–mobile phase (30:70). System suitability was judged from the results obtained for a defined set of injections prior to analyzing the test solutions. The system suitability included resolution between tartrate and citrate peaks in the resolution solution (resolution ≥0.6, defined below), precision for replicate ($n = 5$, relative standard deviation ≤2.0%) injections of mid level standard, accuracy for the analysis of untreated and SPE treated check standard (accuracy within 98.0–102.0% to verify that lot-to-lot variation does not affect recoveries of citrate and acetate), analysis of untreated and SPE-treated reagent water blank (concentrations of peaks, if any, at retention time of citrate or acetate not greater than 0.1% of citrate or acetate in the medical fluid sample, to verify that SPE is not introducing any interfering peak), and method calibration performance ($r \geq 0.995$, and % y-intercept ≤ 5.0). The resolution between

Table 1
Method parameters, data evaluation, and acceptance criteria defined per the validation protocol

Parameter	Data evaluation	Criteria
Specificity	Injections of reagent and placebo blank treated with SPE cartridges were examined for peaks at the retention times of citrate and acetate.	Area of peaks, in reagent water and placebo blank, at the elution times of citrate or acetate are $\leq$ 0.1% of the area of citrate or acetate in check standard, respectively.
Accuracy	The recoveries of SPE treated samples at 80, 100, and 120% were calculated against the untreated samples.	Mean recovery 100.0 $\pm$ 1.0%, defined as ratio of the means $\times$ 100. R.S.D. (relative standard deviation) $\leq$ 2.0% for all experimental results for each test solution
Precision		
Repeatability	R.S.D. values for each of the accuracy study samples were used to calculate the method precision.	R.S.D. $\leq$ 2.0%
Interim	The R.S.D. values for the pooled data from two analysts were used to calculate the method precision.	R.S.D. $\leq$ 2.0%
Linearity of SPE treatment	Peak areas for citrate and acetate for each of the untreated and treated sample preparations were plotted against the concentration.	r^2 not less than 0.998 % *y*-intercept: not more than 5.0 Residual sum of squares: informative
Range	Assay range was where acceptable linearity, accuracy, and precision were obtained.	Passing results for accuracy and precision.
Stability	The recovery of citrate and acetate in SPE treated samples were calculated against the initial concentrations.	Passing results for accuracy and precision.
Ruggedness	Data from two analysts was analyzed for acceptable accuracy, precision, and linearity.	Passing results for accuracy and precision in the pooled data, and passing results for linearity.

citrate and tartrate was calculated from the ratio between height of the tartrate peak from the baseline and height of the valley between the two peaks to the apex of the tartrate peak.

2.4. Preparation of test solutions

Test solutions were prepared to contain citrate and acetate at 80–120% of the nominal concentration of these two analytes in the medical fluid sample. A placebo blank was prepared to contain the matrix without citrate and acetate. Untreated and SPE treated test solutions were then assayed using the ion-exclusion chromatographic procedure.

2.5. Validation procedure

The validation protocol defined the method parameters to be validated, the experiments to be performed, and the acceptance criteria (Table 1). To assess the method ruggedness, two analysts performed the experiments, using two separate instruments and columns.

3. Results and discussion

3.1. Optimization of SPE procedure

SPE devices are available in tube or cartridge format. The tube format requires applying of vacuum, whereas, with the cartridge format, using a luer lock connection, sample placed in a syringe can be pushed through the cartridge. A SPE device in cartridge format was, therefore, selected over that in tube format. Among the various SPE cartridges evaluated, Waters tC_{18} cartridge had narrower particle size distribution (37–55 μm) and 17% carbon load allowing maximum sample loading. Since a reversed-phase medium removed the late eluting peaks, using such cartridge should have the highest removal capacity and be the most rugged. Conditioning of the cartridge with a solvent was essential to avoid appearance of extraneous peaks due to impurities from the cartridge in the chromatographic analysis. In the optimized procedure cartridge was conditioned with 10 ml acetonitrile, with which no extraneous peaks were obtained for the injections of water blank or samples. The effectiveness of this conditioning procedure is verified with every run in the system suitability test.

Experiments were performed to determine the volume of sample that needed to be pushed and discarded through the cartridge prior to collecting an aliquot for the ion-exclusion chromatographic analysis, such that compared with citrate and acetate in untreated solution, full recoveries of citrate and acetate are obtained for the SPE treated solution, and that the cartridge capacity for removal of late eluting peaks is not exceeded. In order for the SPE procedure to be rugged, both of these conditions had to be satisfied. In this experiment, amount of sample pushed and discarded through the cartridge ranged from 2 to 20 ml, in 2-ml increments, and amount of sample collected for the ion-exclusion chromatographic analysis was kept at 1 ml. The obtained data is presented in Fig. 1, which shows that when 6 ml or larger

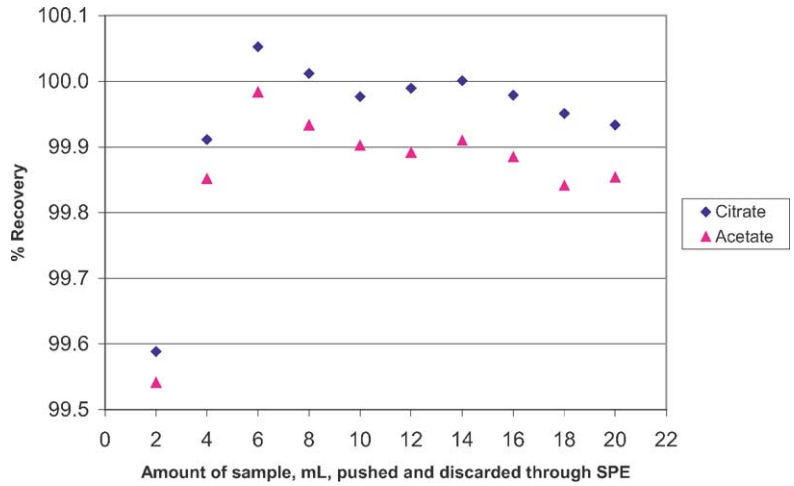

Fig. 1. Recovery of citrate and acetate in medical fluids as affected by amount of sample pushed and discarded through the SPE cartridge. After discarding the mentioned amount of sample, another 1 ml was pushed and collected for the ion-exclusion chromatographic determination of citrate and acetate.

amount of sample was discarded through the cartridge, citrate and acetate were recovered at close to 100%. As evident from the chromatograms obtained, the cartridge capacity in removal of late eluting peaks was not exceeded even when 21 ml sample was pushed through the cartridge. Although the cartridge capacity in removing the late eluting peak remained unknown, the data, therefore, shows that the optimized SPE procedure, consisting of pushing a total of 9 ml sample that is far less than the attempted 21 ml, would not operate at close to the cartridge capacity. The optimized procedure used in routine analysis is described in Section 2.1. To further evaluate the ruggedness of the SPE procedure,

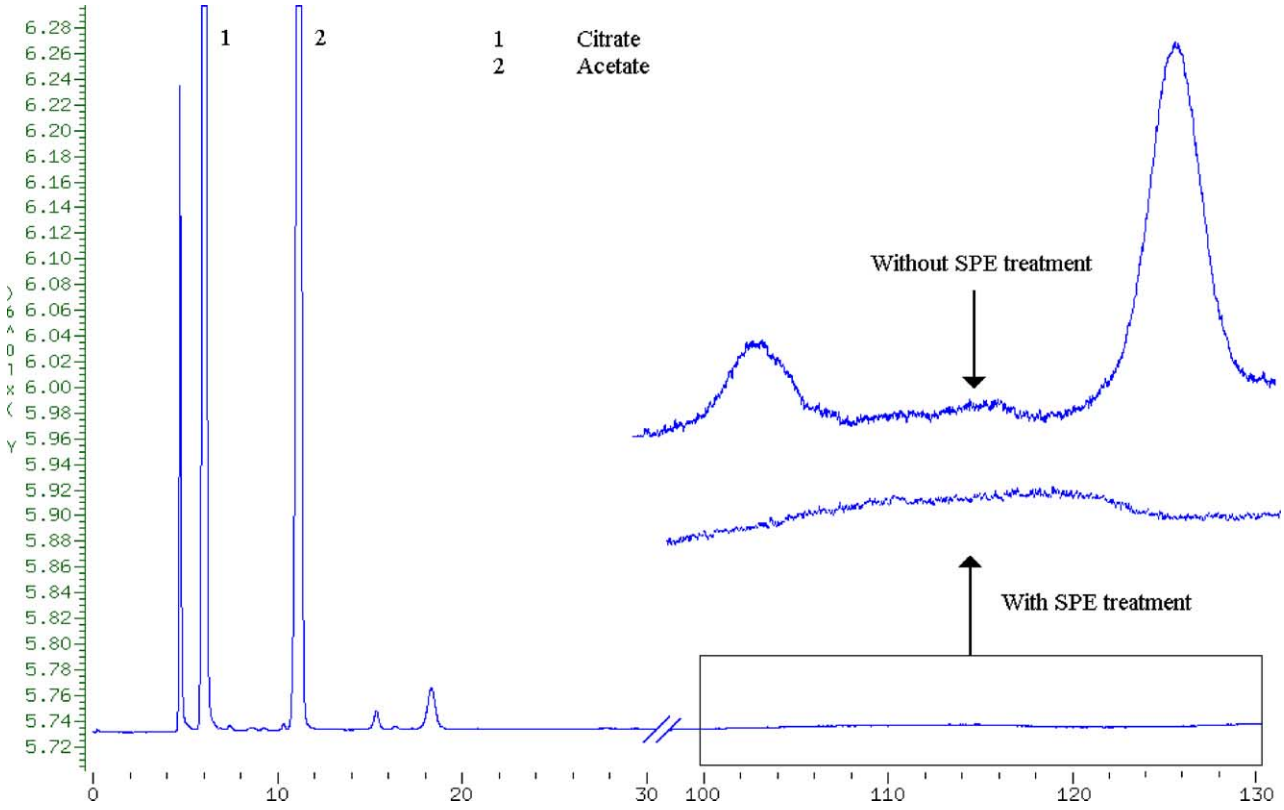

Fig. 2. Chromatogram illustrating removal of late eluting peaks in the medical fluid sample treated with SPE. An 8-ml sample was pushed through the SPE cartridge and discarded. Another 1-ml was then pushed through the cartridge and collected for the ion-exclusion chromatographic analysis. Chromatographic conditions, Mobile phase; 25 mM H_2SO_4 0.8 ml/min, columns; 4.6 mm × 30 mm cation H^+ guard cartridge and 7.8 mm × 300 mm HPX-87H analytical column, 20 μl injection, 60 °C column temperature, and UV detection at 210 nm.

Table 2
Accuracy and precision of the method

Test solution (%)	Analyte	Parameters[a]	Untreated		Treated		Recovery (%)	
			Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
Solution at 80	Citrate (g/l)	Mean	2.56	2.56	2.56	2.56	100.0	99.9
		95% C.I.	0.002	0.002	0.002	0.006		
		R.S.D. (%)	0.02	0.04	0.03	0.09		
	Acetate (g/l)	Mean	3.59	3.58	3.59	3.58	100.0	99.9
		95% C.I.	0.005	0.004	0.007	0.004		
		R.S.D. (%)	0.06	0.04	0.08	0.04		
Solution at 100	Citrate (g/l)	Mean	3.19	3.19	3.19	3.19	100.3	100.2
		95% C.I.	0.004	0.004	0.003	0.004		
		R.S.D. (%)	0.05	0.05	0.04	0.05		
	Acetate (g/l)	Mean	4.46	4.46	4.47	4.47	100.2	100.2
		95% C.I.	0.008	0.005	0.011	0.007		
		R.S.D. (%)	0.07	0.05	0.10	0.06		
Solution at 120	Citrate (g/l)	Mean	3.84	3.83	3.84	3.83	100.0	100.1
		95% C.I.	0.003	0.010	0.004	0.026		
		R.S.D. (%)	0.04	0.10	0.04	0.27		
	Acetate (g/l)	Mean	5.38	5.36	5.38	5.37	100.0	100.2
		95% C.I.	0.003	0.013	0.014	0.034		
		R.S.D. (%)	0.02	0.10	0.10	0.26		

[a] C.I., confidence interval and R.S.D., relative standard deviation. Obtained values are rounded off for presentation purpose.

Table 3
Linearity of response in the SPE-treated samples

Calculation	Analyst I		Analyst II	
	Citrate	Acetate	Citrate	Acetate
Slope	725 521	362 068	728 831	366 534
y-intercept	363 700	132 956	−225 791	−24 020
y-intercept (%)[a]	0.50	0.37	−0.31	−0.07
Residual sum of squares	1.4×10^{11}	4.5×10^{10}	1.25×10^{11}	2.7×10^{10}
Correlation coefficient	0.99989	0.99986	0.99990	0.99992

[a] Calculated vs. the area counts in medical fluid sample containing acetate and citrate at 100%.

a couple of lots of SPE cartridges were evaluated, and the obtained accurate and precise results for citrate and acetate showed that lot-to-lot variation did not affect SPE treatment. With the SPE removal of late eluting peaks, the ion-exclusion chromatography runtime was shortened from 180 to 35 min (Fig. 2). Quadruplicate injections of SPE treated samples, with a runtime of 35 min, were made, and it was confirmed that the SPE fully removed the late eluting peaks since there were no indications of carried over peaks, potentially resulting from inadequate SPE removal, in these multiple injections.

3.2. Validation of the ion-exclusion chromatographic method with SPE cleanup

Two analysts performed experiments as described in Table 1. Both analysts passed the system suitability requirements (data not presented). The validation results obtained are discussed below.

Table 4
Stability of SPE-treated samples

Time (h)	Parameter	Citrate	Acetate
0	Average ($n = 3$)	3.22	4.51
	R.S.D. (%)	0.06	0.05
	Accuracy[a]	100.0	100.0
24	Average ($n = 3$)	3.22	4.50
	R.S.D. (%)	0.02	0.03
	Accuracy	99.9	99.9
48	Average ($n = 3$)	3.23	4.51
	R.S.D. (%)	0.02	0.03
	Accuracy	100.1	100.1
	Overall precision		
	Average	3.22	4.51
	95% C.I.	0.003	0.003
	R.S.D. (%)	0.11	0.09

[a] Accuracy calculated as: [(average concentration at the specified time)/(average concentration at time zero)] × 100.

Table 5
Pooled data on accuracy and precision showing method ruggedness[a]

Test solution (%)	Analyte	Parameters[b]	Untreated	Treated	Recovery (%)
Solution at 80	Citrate (g/l)	Mean	2.56	2.56	99.9
		95% C.I.	0.003	0.004	
		R.S.D. (%)	0.11	0.14	
	Acetate (g/l)	Mean	3.58	3.58	100.0
		95% C.I.	0.003	0.005	
		R.S.D. (%)	0.09	0.14	
Solution at 100	Citrate (g/l)	Mean	3.19	3.19	100.2
		95% C.I.	0.002	0.002	
		R.S.D. (%)	0.05	0.05	
	Acetate (g/l)	Mean	4.46	4.47	100.2
		95% C.I.	0.002	0.004	
		R.S.D. (%)	0.05	0.08	
Solution at 120	Citrate (g/l)	Mean	3.84	3.84	100.1
		95% C.I.	0.010	0.010	
		R.S.D. (%)	0.25	0.25	
	Acetate (g/l)	Mean	5.37	5.37	100.1
		95% C.I.	0.014	0.013	
		R.S.D. (%)	0.26	0.24	

[a] Results obtained from two analysts for the analyses of untreated and SPE treated samples are pooled.
[b] Obtained values are rounded off for presentation purpose.

3.2.1. Specificity

Area counts for citrate and acetate peaks in, untreated and treated, distilled water and placebo blank samples were not greater than 0.1% of the area counts for these peaks in untreated check standard (data not presented). The data illustrated that the SPE cleanup procedure is specific for the quantitation of citrate and acetate.

3.2.2. Accuracy and precision

Accuracy and precision data for the two analysts are summarized in Table 2. The data demonstrated accuracy of the method with recovery of citrate and acetate ranging from 99.9 to 100.3%. The method was precise with R.S.D. values ranging from 0.02 to 0.27%. Exemplary chromatogram for the treated medical fluid sample at 100% nominal concentration is presented in Fig. 2. The pooled data from two analysts, obtained on two separate instruments with two different columns, gave R.S.D. values ranging from 0.05 to 0.26%, and the data illustrated interim precision of the method.

3.2.3. Linearity

Citrate and acetate determinations in the SPE treated medical fluid sample are linear, in the range of 80–120% concentration, with correlation coefficient of > 0.998 and y-axis intercept of $\leq 0.5\%$ (Table 3). Based on the data obtained for accuracy, precision, and linearity, it was concluded that the SPE treatment covered a range of 80–120% concentration of citrate and acetate in the formulation.

3.2.4. Stability of SPE-treated samples

The results for stability of SPE-treated samples are presented in Table 4. Citrate and acetate values at 24 and 48 h were 99.9 and 100.1% compared with those at 0 h. The R.S.D. values at each time point ranged from 0.02 to 0.06% and overall R.S.D. values were 0.11 and 0.09% for citrate and acetate, respectively. Hence, the SPE treated samples are stable for at least 48 h.

3.2.5. Ruggedness

Pooled data on accuracy and precision using determined values are presented in Table 5, which shows that the method met the requirements for accuracy and precision. Both analysts also obtained response linearity in the range of 80–120% of the nominal concentration for citrate and acetate (Table 3). The method is, therefore, rugged.

4. Summary

The ion-exclusion chromatography method for the determinations of citrate and acetate with SPE cleanup enabled shortened runtime from 180 to 35 min. Results of method validation showed that the method is valid for the intended purpose since the results, for each parameter, met the acceptance criteria set forth in the validation protocol. The method has been routinely used in the manufacturing environment for the analysis of medical fluid samples.

References

[1] J. Weiss, in: Ion Chromatography, VCH, Weinheim, 2nd ed., 1995.
[2] M.J. Del Nozal, J.L. Bernal, P. Marinero, J.C. Diego, J.I. Frechilla, M. Higes, J. Llorente, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 3197.
[3] M. Schiller, H. Heydt, F. Marz, P. Schmidt, J. Chromatogr. A 968 (2002) 101.

[4] A. Schneider, V. Gerbi, M. Redoglia, Am. J. Encol. Viticult. 38 (1987) 151.
[5] J. Chen, J. Chromatogr. A 739 (1996) 273.
[6] K. Pyrzynska, E. Pobozy, Crit. Rev. Anal. Chem. 32 (2002) 227.
[7] L. Snyder, J. Kirkland, J. Glajch, in: Practical HPLC Method Development, Wiley, New York, 2nd ed., 1997, Chapter 4.
[8] SPE Reference Manual and Users Guide, Phenomenex Torrance, CA, 2002.
[9] L. Bhattacharya, in: 15th International Ion Chromatography Syposium, Baltimore, 2 October 2002.
[10] ICH Guideline Q2A, Validation of Analytical Procedures: Definition and Terminology (CPMP III/5626/94), Geneva, March 1995.
[11] ICH Guideline Q2B, Validation of Analytical Procedures, Methodology (CPMP/ICH/281/95), Geneva, Switzerland, November 1996.
[12] Validation of Compendial Methods, The United States Pharmacopeia, XXVI, USP Convention, Rockville, MD, 2002, pp. 2439–2442.