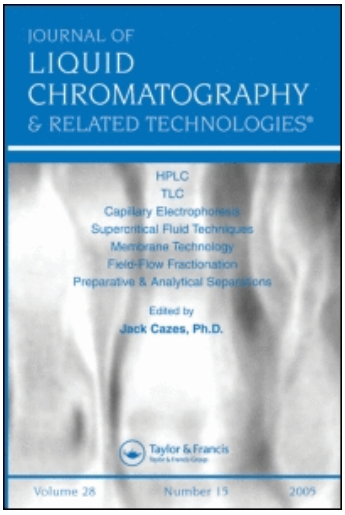


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic Analysis of Residual Acetate in Bulk Drugs

M. M. See[a]; R. Thompson[a]; N. Grinberg[a]; H. J. Perpall[a]; G. Bicker[a]; P. Tway[a]
[a] Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

To cite this Article See, M. M. , Thompson, R. , Grinberg, N. , Perpall, H. J. , Bicker, G. and Tway, P.(1995) 'Chromatographic Analysis of Residual Acetate in Bulk Drugs', Journal of Liquid Chromatography & Related Technologies, 18: 1, 137 — 154
To link to this Article: DOI: 10.1080/10826079508009227
URL: http://dx.doi.org/10.1080/10826079508009227

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(1), 137-154 (1995)

CHROMATOGRAPHIC ANALYSIS OF RESIDUAL ACETATE IN BULK DRUGS

MARJORIE M. SEE, R. THOMPSON, N. GRINBERG*, HOLLY J. PERPALL, G. BICKER, AND PAT TWAY
Merck Sharp & Dohme Research Laboratories
R80Y-115, P.O. Box 2000
Rahway, New Jersey 07065-0914

ABSTRACT

An ion chromatographic method for the determination of the residual acetate in bulk drug was developed. The drug was MK0476, an LTD_4 antagonist. The compound also has a carboxyl functionality, which would interfere with the detection of the acetate ion. A solid phase extraction through a Sep-Pak cartridge was pursued for the removal of MK0476 from the matrix. Since the analyte does not have a chromophore, a mobile phase containing trimesic acid facilitated its detection by indirect photometric detection. The separation was performed on a polymeric strong anion exchange column. The influence of pH, concentration of trimesic acid, and temperature were studied. Chloride ion was found to be a contaminant that was interfering in the analysis. To improve the separation between chloride and acetate ions, methanol was added to the mobile phase, leading to complete separation between the two species. Recovery of the acetate ion was determined as 92.3%. The method was applied to real samples with good results. It was shown to be sensitive for the determination of less than 0.001 mg/ml of acetate with a linear range of 0.00036 to 0.074 mg/ml.

* Author to whom the correspondence should be addressed

I. INTRODUCTION

The use of ion chromatography for the analysis of inorganic and organic ions has shown an exponential increase since the mid 1970's due to the implementation of suppressor systems [1]. Prior to this time, the concept of ion exchange chromatography was well known [2-6], but its application was hindered by the lack of a good detector. Many ions were unsuitable for UV detection due to lack of chromophores, and the use of conductivity detection was rendered impossible due to the high background conductivity of the eluents used. Suppressor systems removed most of the background conductivity by exchanging ions such as sodium and nitrate by protons and hydroxide ions respectively. With the introduction of suppressor systems ion chromatography using conductivity detection grew rapidly and at present conductivity is still the most popular choice of detection used.

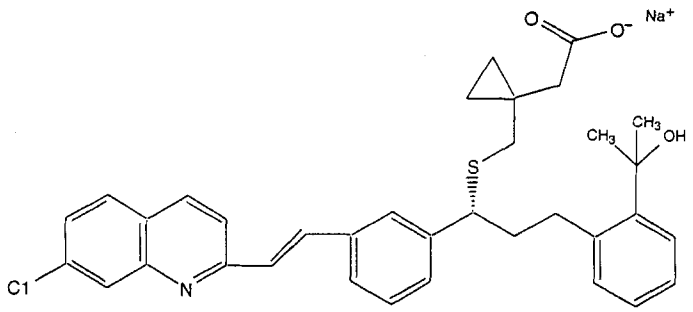
Several alternate detection systems have been developed since the introduction of suppressor systems. They include direct conductivity detection using low conductivity eluents [7], UV detection following post or pre-column derivatization [8], amperometric detection [9,10], and indirect photometric detection [11-13].

Indirect photometric detection (IPD) or "vacancy detection" offers an alternative that is as sensitive as conductivity detection [14,15], does not require derivatization, and utilizes standard HPLC pumps and detectors. Aromatic carboxylic acids are typically used as additives to

the mobile phase because of their high absorptivity in the UV-Vis range. At a chosen wavelength where the eluent absorbs strongly and the analyte does not, the presence of the analyte in the detector cell will result in a decrease in absorbance. This decrease will be manifested as a negative peak. Many inorganic and organic ions have been analyzed using IPD [15-18].

In a previous paper, our laboratory showed the effectiveness of using trimesic acid as an eluent for the separation of some organophosphonates [19]. The work described in this paper is an extension of the previous study dealing with the quantitation of trace acetate ion in the presence of the bulk drug MK0476. The compound, MK0476, is a molecule with a carboxyl functionality, Fig. 1.

This drug is a potent receptor antagonist of leukotriene D_4 (LTD_4) which is being developed as a therapeutic agent for bronchial diseases such as asthma [20-22]. In the penultimate step of its synthesis,



MK0476

Fig. 1. Structure of MK0476.

MK0476 is obtained as a dicyclohexyl amine (DCHA) salt. Addition of acetic acid to the reaction mixture produces an exchange of the DCHA leading to the free acid dissolved in the organic layer. An ethanolic solution of sodium hydroxide is added to convert the MK0476 free acid to the sodium salt which then precipitates out. The salt is subsequently washed with acetonitrile to remove residual acetic acid and is then analyzed for trace acetate by ion chromatography.

II. EXPERIMENTAL

Sample preparation.

Ten batches of samples were prepared by weighing 100 mg of MK0476 sodium salt that were then introduced into 10 ml volumetric flasks. Five ml of deionized water were added and the volumetric flask was introduced into an ultrasonic bath and sonicated until the compound was completely dissolved. The flask was then diluted to volume with water. Batch MO35 was used for the recovery experiment. An aliquot was removed from this sample for sample treatment (as described in the next section). Then, 2 ml of 0.006 mg/ml acetate standard was spiked into 2 ml of the sample solution. The solution obtained was also subjected to the same sample treatment as the unspiked solution.

Sample treatment.

To remove MK0476 from the samples, each sample was passed

through a Sep Pak C18 cartridge (Waters, Marlborough, MA) that was previously conditioned in the following manner. First, the cartridge was washed with 10 ml of methanol followed by 20 ml of water deionized water. Then the sample solutions and the solutions spiked with acetate were passed through a cartridge. The first 2 ml were then discarded. Twenty-five microliters of sample were injected into the HPLC system.

HPLC conditions.

An HP1050 HPLC system equipped with an autosampler and a variable wavelength detector was used for the analysis of the residual acetate in the MK0476 sodium salt.

The stationary phase, manufactured by Hamilton under the trade name PRP-X-100, consisted of a polymeric strong anion exchange packed in a 25 cm x 0.4 cm I.D. column. The column was maintained at constant temperature of 30 °C.

The optimal mobile phase consisted of a mixture of an aqueous solution of 0.5 mM trimesic acid. The pH was adjusted to 5.0 with LiOH, and methanol at 90:10 (v/v) ratio.

The analyte was detected by UV at 254 nm. Due to the negative peaks obtained with the indirect photometric detection method the polarity of the detector was reversed such that the peaks were inverted and facile integration of the peaks was achieved.

III. RESULTS AND DISCUSSION

To analyze residual acetate in MK0476 final product a strong anion exchange column was selected. MK0476 has a carboxylic functionality that can interact with the stationary phase. Under our chromatographic conditions MK0476 elutes as a large peak close to the void volume. Consequently, a solid phase extraction was necessary to remove the compound from the system prior to the chromatographic analysis.

Acetate is an analyte that has very little absorbance in the UV-Vis region, and the detection of trace amounts of this analyte thus represents a challenge. Indirect photometric detection was chosen to analyze the acetate ion. The method is based on the use of a mobile phase that has a high absorbance at a particular wavelength; at the same time the analyte presents no or minimal absorbance at that same wavelength. Upon equilibration of the stationary phase with the mobile phase ions, the detector senses a strong steady absorbance. When the analyte ion is injected and eventually eluted from the stationary phase, its transparency results in a localized region of lower absorbance and is reflected in the detector as a negative analyte peak.

Trimesic acid is a benzene tricarboxylic acid. It absorbs strongly at 254 nm while acetate ion does not, and is therefore suitable for indirect photometric detection. Injection of a solution of acetate into the chromatographic system produces a negative peak. The polarity of the detector can be inverted to obtain a positive peak.

Several parameters, such as the concentration of trimesic acid in the mobile phase, the pH of the mobile phase, the concentration of methanol in the mobile phase and the temperature were varied to optimize the method.

Influence of the pH of the mobile phase on the peak parameters. Trimesic acid is a tricarboxylic acid and consequently it has three pK values of 3.1, 3.9 and 4.7. It is therefore expected that the net charge of the acid, and consequently its displacing power, will vary with the changes in the pH of the mobile phase. Consequently, the retention of acetate on the anion exchange stationary phase will be strongly influenced. Thus, the pH of the mobile phase was varied between 4.0 and 6.25 as follows: 4.0, 4.25, 4.6, 5.0 and 6.25. As expected, the k' of the analyte is strongly affected by the pH changes. Between 4.0 and 4.25, it is almost constant, and decreases sharply at the rest of the pH intervals studied (Fig. 2).

Since the present study deals with the analysis of trace amounts of acetate ions, the method should be optimized such that the half peak width ($W_{1/2}$) should be at a minimum. This optimization will insure lower detection limits. The influence of the pH on the $W_{1/2}$ is presented in Fig. 3. From this data the minimum $W_{1/2}$ was obtained at pH 5. Therefore, in the following experiments the pH was maintained at this value.

Influence of the concentration of trimesic acid. The concentration of the trimesic acid in the mobile phase was varied between 0.25 mM

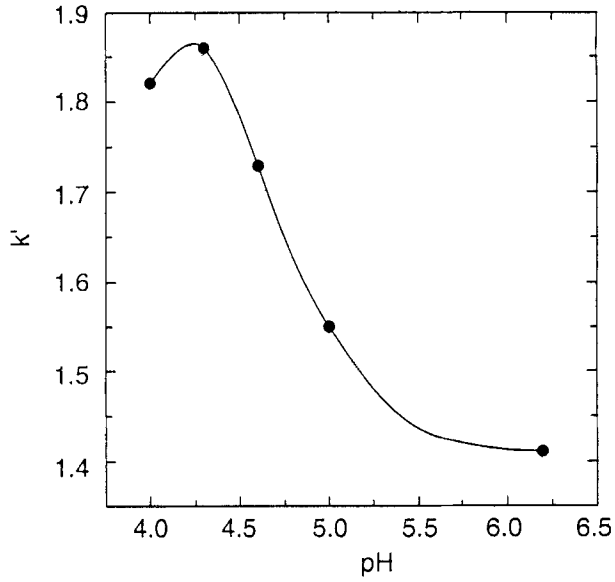


Fig. 2. Influence of pH on the k'.

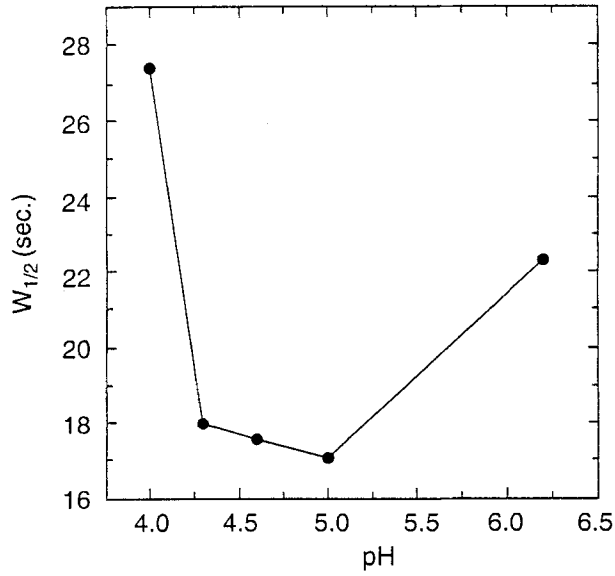


Fig. 3. Influence of pH on the $W_{1/2}$.

and 1.5 mM as follows: 0.25, 0.5, 1.0 and 1.5 mM. The influence of the concentration of trimesic acid on the k' of the acetic acid is presented in Fig. 4. Upon increasing the trimesic acid concentration from 0.25 mM to 1.5 mM the k' values decreases steadily.

The influence of the concentration on $W_{1/2}$ is presented in Fig. 5. The increased concentration of the additive into the mobile phase produces a decrease in the mass transfer of the acetate ion. This will lead to an increase in $W_{1/2}$ up to a concentration of 1 mM, decreasing after this concentration.

The increase of the concentration of trimesic acid into the mobile phase also has a negative effect on the detection of the acetate ion. The results are presented in Fig. 6. From 0.25 mM to 0.5 mM an increase in the area counts for the acetate is observed. A further increase of concentration of the additive into the mobile phase produces a decrease in area counts. Based on these results the concentration of trimesic acid in the mobile phase was maintained constant at 0.5 mM for further optimization studies.

Influence of the temperature. The influence of temperature on the k' is presented in Fig. 7. Upon increasing the temperature, an increase in k' is observed. Such behavior is due to a shift in the partition equilibrium of trimesic acid toward the mobile phase, leaving more sites in the stationary phase to interact with the acetate ion. Due to an increased interaction of the acetate ion with the stationary phase an

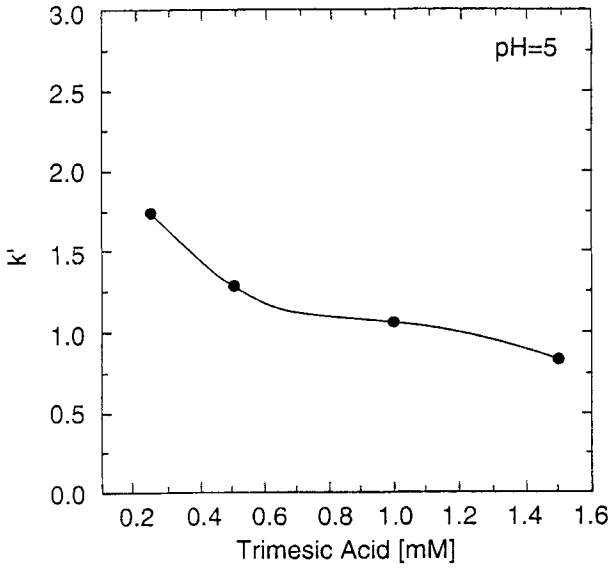


Fig. 4. Influence of the concentration of trimesic acid on the k' values.

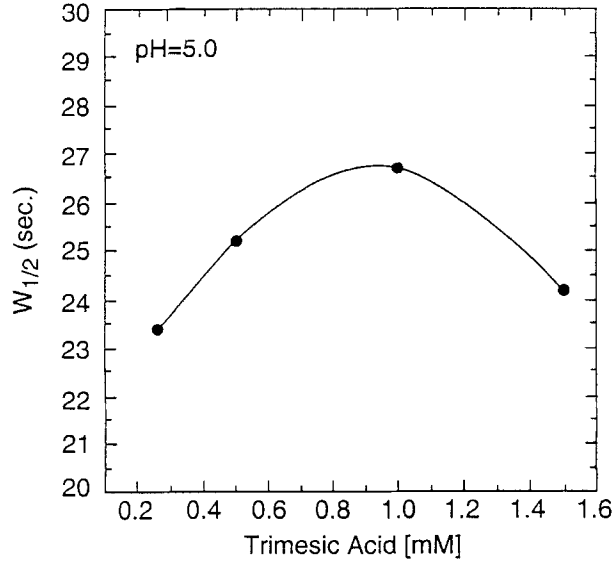


Fig. 5. Influence of the concentration of trimesic acid on the $W_{1/2}$.

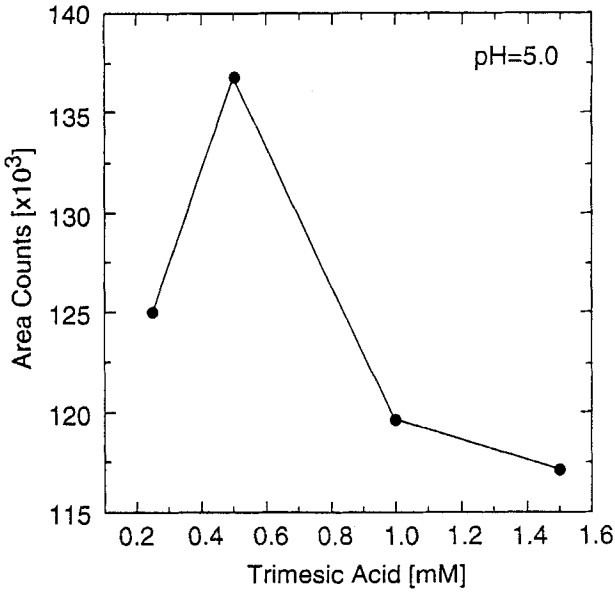


Fig. 6. Influence of the concentration of trimesic on the area counts.

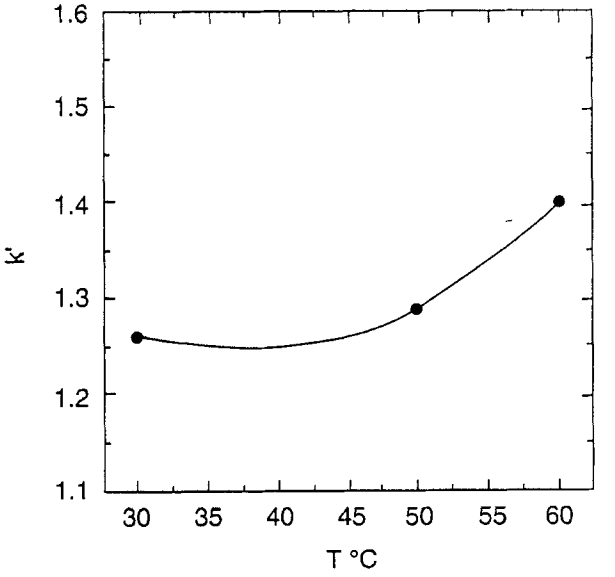


Fig. 7. Influence of the temperature on the k'.

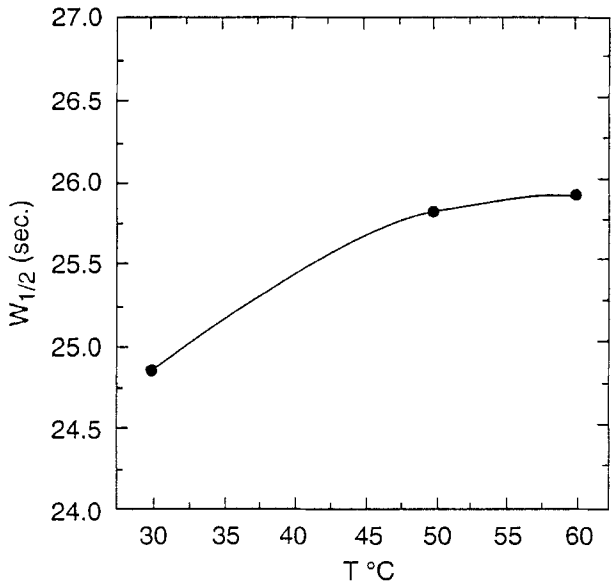


Fig. 8. Influence of the temperature on the $W_{1/2}$.

increase of $W_{1/2}$ was also observed (Fig. 8). Therefore, 30 °C was chosen as the temperature for the subsequent experiments.

Influence of the concentration of methanol in the mobile phase.

Chloride was observed as an impurity in all the standard solutions, as well as in the samples. Its presence is due to inherent laboratory contamination. Special care was placed on improving the resolution between the acetate ion and chloride. With no organic modifier the two peaks were partially separated. It is known that decreasing the polarity of the mobile phase (by adding an organic modifier to the mobile phase) will lead to an increase of the ionic character of the stationary phase [18]. The interaction of the analyte with the

stationary phase will then be enhanced. Acetonitrile could not be used because it contains trace levels of acetic acid. Consequently, methanol was used as an organic modifier. An increase in the concentration of methanol in the mobile phase produced an increased resolution between the two ions (Fig. 9). Upon increasing the methanol concentration, a slight decrease in the retention time of acetate and an increase in the retention time of chloride was observed. The observed decline in the retention of acetate ion can be attributed to a slight elevation in its solubility with increasing methanol. The increase retention of chloride is due to an enhanced polarity of stationary phase relative to the mobile phase. Similar results were obtained by Walker et al. [18].

Determination of the linear dynamic range of the detector. To determine the linear dynamic range of the detector, a series of solutions of sodium acetate in a range of concentrations varying from 0.00036 mg/ml to 0.07416 mg/ml was prepared and injected into the HPLC system. The results are presented in Fig. 10. A r^2 of 0.9996 was obtained, which is sufficient for the range of concentrations studied. The method was capable of detecting less than 0.00036 mg/ml.

Determination of the recovery through the Sep-Pak cartridges. An important step in the quantitation of the real samples of MK0476 consists in determining whether the acetate ion is retained on the Sep-Pak cartridge during the sample preparation process. Standard

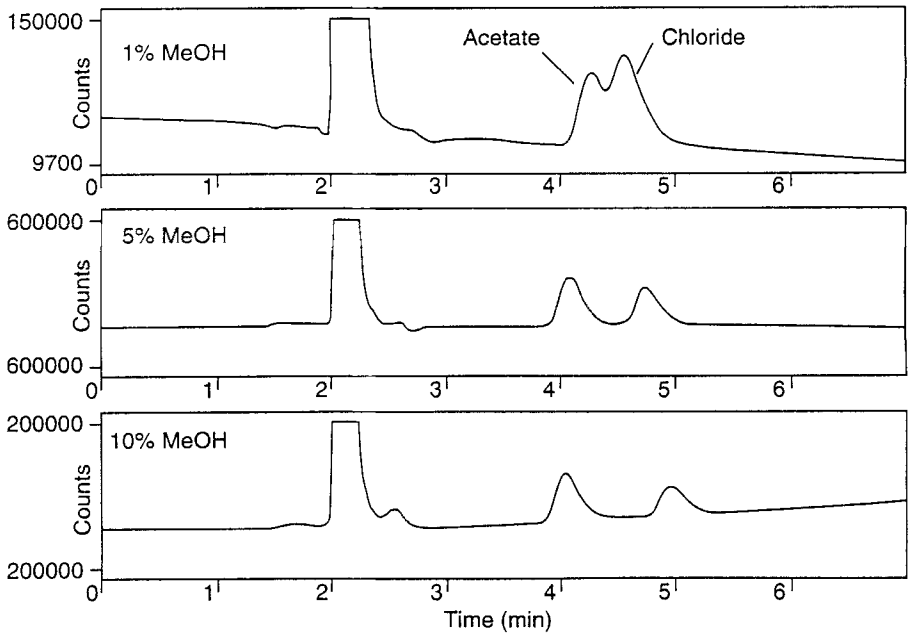


Fig 9. Influence of methanol concentration on the separation of chloride and acetate.

solutions of acetate in increasing concentrations were prepared. Half of each solution was subjected to HPLC analysis without further treatment. The remaining solutions were passed through a Sep-Pak cartridge before injection into the HPLC system. A plot was constructed to determine the recovery. Area counts obtained from the HPLC analysis without filtration were plotted on the X axis, and area counts from the analysis with filtration through the Sep-Pak cartridge were plotted on the Y axis. A linear plot was obtained (Fig. 11) with a slope of 0.93977 and a $r^2 = 0.9992$, suggesting that no or few acetate ions were retained on the cartridge.

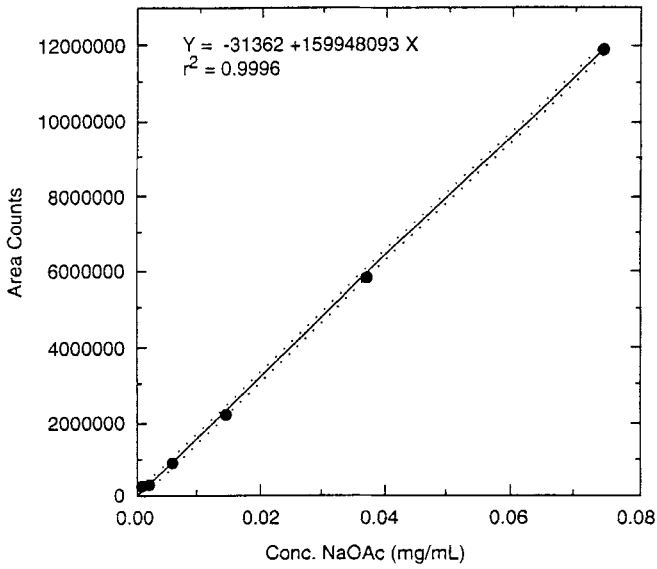


Fig. 10. Linear regression for the sodium acetate standards at 95% confidence limit.

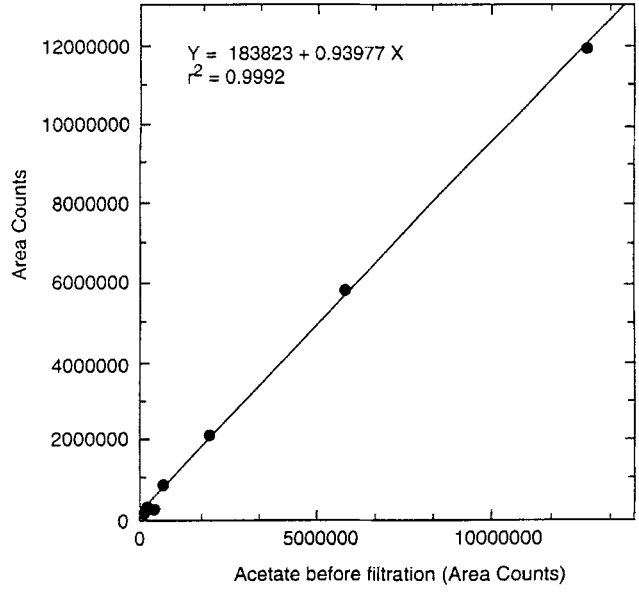


Fig. 11. Recovery of acetate through Sep-Pak cartridges.

Table 1

Results of the Analysis of Residual Acetate in MK0476*

MK0476 Batch #	%NaOAc	%RSD
MO22	0.079	0.62
MO23	0.034	2.68
MO24	0.054	1.24
MO25	0.066	0.58
MO26	0.050	0.97
MO27	0.019	1.74
MO29	0.020	7.66
MO33	0.074	0. 29
MO34	0.089	0.21
MO35	0.031	1.15

*Each sample was analyzed in triplicate

Results of final product batches. Several samples representing final product batches were analyzed for the residual acetate. The results are presented in Table 1. To determine the influence of the sample matrix on the sample recovery during the sample treatment procedure, batch

MO35 was spiked with 0.006 mg/ml solution acetate standard. The sample, the spiked sample and the 0.006 mg/ml acetate solution were subjected to the HPLC analysis according to the experimental section. A recovery of 92.3% was obtained.

III. CONCLUSIONS

A new method for the analysis of residual acetate was developed. The sample preparation provided for the removal of the final product in a way that did not interfere with the analysis of the residual acetate. The method has been demonstrated to be sensitive for the determination of less than 0.001 mg/ml of acetate with a linear range of 0.00036 to 0.074 mg/ml.

REFERENCES

1. H. Small, T.S. Stevens, and W.C. Bauman, *Anal. Chem.* , 47, 1801- 1809, 1975.

2. F.H. Spedding, A. F. Voight, E.M. Gladrow, and N.R. Sleight, J. Am. Chem. Soc. , 69, 2777-2781, 1947.

3. J.A. Marinsky, L. E. Glendenin , and C.D. Coryell, J. Am. Chem. Soc. , 69, 2781-2786, 1947.

4. E.R. Thompkins, and S.W. Mayer, J. Am. Chem. Soc. , 69, 2859-2866, 1947.

5. S. W. Moore and W. H. Stein, J. Biol. Chem., 192, 663-681, 1951.

6. P.B. Hamilton, Anal. Chem. 32, 1779-1781, 1960.

7. D. J. Gjerde, J.S. Fritz, and G. Schmukler, J. Chromatogr., 186, 509-519, 1979.

8. J. Weiss, Handbook of Ion Chromatography, Dionex Corporation, Sunnyvale, California, 148-150, 1986.

9. R.D. Rocklin and E.L. Johnson, Anal. Chem., 55, 4-7, 1983.

10. P. Edwards and K. Haak, Amer. Lab, April 1983.

11. M. Denkert, L. Hackzell, G. Schill, and E. Sjogren, J. Chromatogr., 218, 31-43, 1981.

12. H. Small and T. E. Miller, Anal. Chem., 54, 462-469, 1982.

13. R. A. Cochrane and D. E. Hillman, J. Chromatogr., 392-394, 1982.

14. S. Wilson and E. Yeung, Anal. Chim. Acta, 157, 53- , 1984.

15. N. Chauret, and J. Hubert, J. Chromatogr., 329-338, 1989.

16. T. A. Walker, T.U. Huo, and N. Akbari, J. Liq. Chromatogr., 14(7), 1351-1366, 1991.

17. S. Motomizu, M. Oshima, and T. Hironaka, Analyst, 116, 695-700, 1991.

18. T. A. Walker, T.U. Huo, and N. Akbari, J. Liq. Chromatogr., 12(7), 1213-1230, 1989.

19. R. Thompson, N. Grinberg, H. Perpall, G. Bicker, and P. Tway, J. Liq. Chromatogr., 17(11), 2522-2531, 1994.

Received: August 29, 1994
Accepted: September 7, 1994