DETERMINATION OF ACETATE IN PHARMACEUTICAL PRODUCTS BY HPLC†

Svjetlana Mihaljica, Jelena Trbojević
Institute of Pharmacy of Serbia, Vojvode Stepe 458, Belgrade, Yugoslavia

Marija Mašković
Institute of Pharmacy of Serbia, Vojvode Stepe 458, Belgrade, Yugoslavia

†This paper is based on a presentation at The Fourth Slovenian Symposium on Separation Techniques meeting held in Novo Mesto, Slovenia during October 3-4th, 2002.

Received 16-12-2002

Abstract

HPLC method, using anion exchange column and UV/Vis detector, for identification and determination of acetate in medicinal products is described in this study. SUPELCOSIL SAX1 strong anion exchange column has ion exchange character. In the ion exchange mode, different pH and ion strength will influence the retention time of acetate. Increasing the ionic strength will reduce its retention time. Changing the pH will control the protonation state of the analyte.

Acetate has been found in those products as salt L-Lysinacetate. Validation of the method establishes that its performance characteristics (figures of merits, quality of parameters) are adequate for the intended use. It entails the evaluation of number of parameters, such as selectivity, linearity, accuracy, specificity, precision (repetability and reproducibility), sensitivity, detection and determination limits and robustness.

Introduction

Acetate was usually determined by titration with sodium hydroxide using phenolphtalein as indicator,[1,4] or by non-aqueous titration with perchloric acid using *p*-naphtholbenzein as indicator.[2]

In the meantime, the examinations of the content of acetate in pharmaceutical products as solutions for infusion by those methods had given the wrong results. Contents of secondary compounds, such as amino acids, in those products inhibit the accuracy of determining assay. There are references in the literature describing the identification,[3] and determination of acetate in drug formulations using GC,[2] HPLC,[5,7] and Capillary Electrophoresis.[6]

The object of this study was to develop HPLC method, using anion exchange column and UV/Vis detector, for identification and determination of acetate in *Aminoven* and *AMINO-MEL "nephro"* solutions for infusion. Acetate has been found in those products as salt L-Lysinacetate. The proposed method needs to be fast, reliable, reproductive and used for daily routine laboratory work.

Results and discussion

The proposed HPLC method was developed and validated and could be used for identification and determination of acetate. The chromatographic process separates the two components (L-Lysine and acetate) from each other as well as from the excipients.

SUPELCOSIL SAX1 strong anion exchange column has ion exchange character. In the ion exchange mode, different pH and ion strength will influence the retention time of acetate. Increasing the ionic strength will reduce its retention time. Changing the pH will control the protonation state of the analite.

Acetate was identified by comparison of the retention times of the peak attributed to acetate in the sample solutions to that of standard solution. Quantification was achieved by comparing the peak area of acetate in sample with the corresponding peak in standard.

A typical chromatograms containing L-Lysine and acetate were showed in Figure 1, Figure 2 and Figure 3.

Efficiency, peak asymmetry and resolution establish chromatographic conditions that ensure the system in a manner suitable to carry out the analysis.

Efficiency was confirmed calculating the number of theoretical plates (N) for the chromatographic column using the acetate peak. The number of theoretical plates was 5827 (>2000).

Peak asymmetry was confirmed calculating peak asymmetry (T) for the acetate peak. Peak asymmetry was 1.5 (<=1.5).

Resolution was confirmed calculating the resolution (R) between the L-Lysine and acetate peaks. The resolution was 14.2.

Estimates of limits of detection and quantitation for acetate were obtained from the linearity data. Based on the average of the three founded peak area values, limits of detection is $8.47 \cdot 10^{-5}$ mol/L and limits of quantification is $1.69 \cdot 10^{-4}$ mol/L.

Solutions of acetate encompassing 80-120% of the target level ($1.7 \cdot 10^{-3}$ mol/L) were analyzed in absence of excipient blend. A plot of peak area versus concentration in absence of excipient blend is shown in Figure 4. The HPLC method was shown to be linear over the concentration ($1.36 \cdot 10^{-3}$–$2.04 \cdot 10^{-3}$ mol/L), y = 1E+06 × -99767, R^2=0.9997.

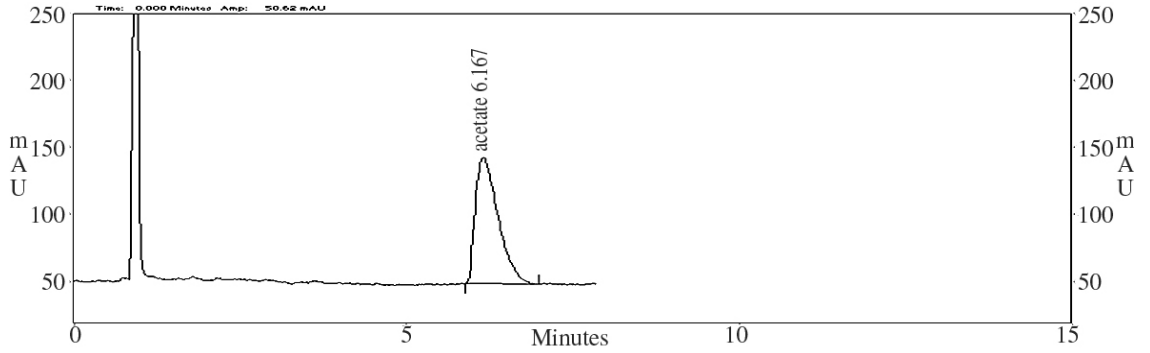

Figure 1. A typical HPLC Profile of L-Lysinacetate Working Standard Solution.

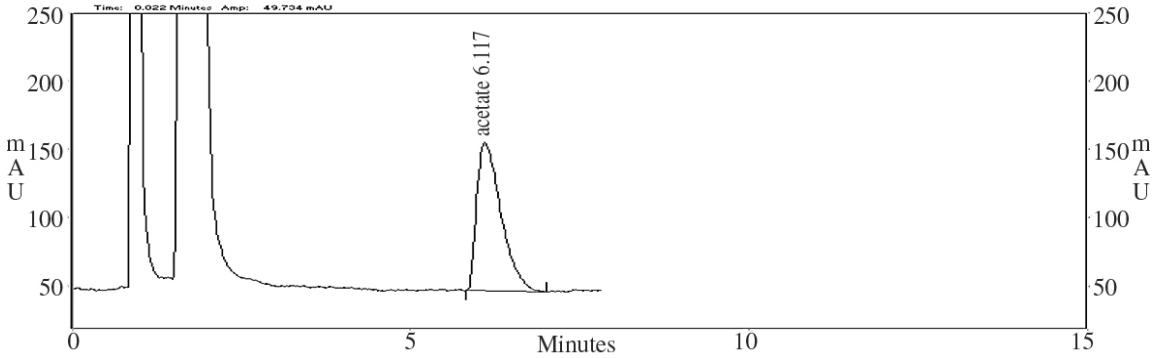

Figure 2. Chromatogram of Sample Solution *AMINO-MEL "nephro"* (batch no. B120024).

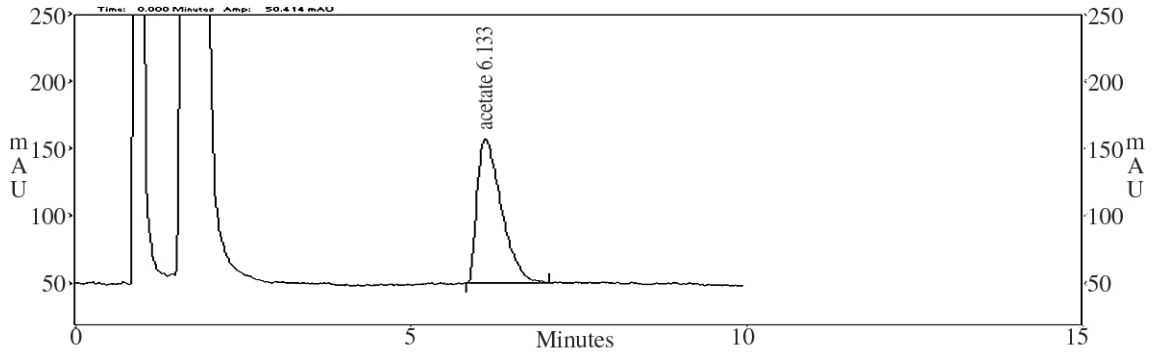

Figure 3. Chromatogram of Sample Solution *Aminoven 5 %* (batch no. MG 1596).

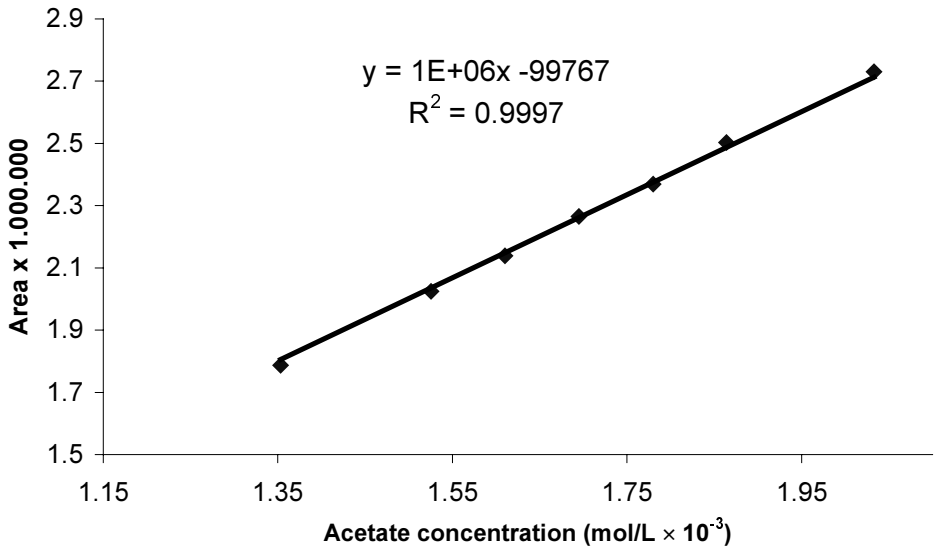

Figure 4. Peak Area vs. Concentration for Acetate in absence of placebo matrix.

The HPLC system precision was determined at the assay target lvel by performing seven replicate injections of a solution containing $1.7 \cdot 10^{-3}$ mol/L acetate. A relative standard deviation of 0.99% based on peak area was obtained. The data are presented in Table 1.

Table 1. Acetate System Precision.

Replicate $1.7 \cdot 10^{-3}$ mol/L	Peak Area
1	2283349
2	2238358
3	2250278
4	2273199
5	2239980
6	2268940
7	2297456
Mean:	2264509
Standard Deviation:	22448.7
% Relative Standard Deviation:	0.991

The accuracy of the method was tested by measuring the recovery of an acetate. Nine determinations for concentration 80%, 100% and 120% of the target level ($1.7 \cdot 10^{-3}$ mol/L) utilized, with quantification by peak area. The average recoveries were 99.7%,

with a relative standard deviation of 1.60%, for concentration $1.36 \cdot 10^{-3}$ mol/L (80%), 99.6%, with a relative standard deviation of 0.69%, for concentration $1.7 \cdot 10^{-3}$ mol/L (100%) and 99.3%, with a relative standard deviation of 1.27%, for concentration $2.04 \cdot 10^{-3}$ mol/L (120%). The data are presented in Table **2**.

Table 2. Accuracy- Recovery of Acetate.

mol/L Added	mol/L Found	% Recovery
$1.36 \cdot 10^{-3}$	$1.34 \cdot 10^{-3}$	98.46
$1.36 \cdot 10^{-3}$	$1.35 \cdot 10^{-3}$	99.13
$1.36 \cdot 10^{-3}$	$1.38 \cdot 10^{-3}$	101.50
Mean:	99.7	
Standard deviation:	28306.4	
% Relative Standard Deviation:	1.60	
$1.7 \cdot 10^{-3}$	$1.70 \cdot 10^{-3}$	100.02
$1.7 \cdot 10^{-3}$	$1.70 \cdot 10^{-3}$	99.97
$1.7 \cdot 10^{-3}$	$1.68 \cdot 10^{-3}$	98.81
Mean:	99.6	
Standard deviation:	15377.5	
% Relative Standard Deviation:	0.69	
$2.04 \cdot 10^{-3}$	$2.05 \cdot 10^{-3}$	100.67
$2.04 \cdot 10^{-3}$	$2.05 \cdot 10^{-3}$	99.04
$2.04 \cdot 10^{-3}$	$2.00 \cdot 10^{-3}$	98.19
Mean:	99.3	
Standard deviation:	34208.3	
% Relative Standard Deviation:	1.27	

Conclusions

The method for determining the content of acetate according to the above mentioned subject is validated. The specificity, accuracy, linearity, and the precision of the method were tested. In addition, the quantification and detection limit of acetate were determined.

Based on the validation data, the described procedure is suitable for the identification and determining the content of acetate in *Aminoven* and *AMINO-MEL "nephro"* solutions for infusion.

Experimental

HPLC analyses were perfomed with a GBC system LC 1120 HPLC Pump equipped with an LC 1210 UV/Vis detector and Rheodyne injector valve (20μL sample loop). Separation was made on a Supelcosil LC-SAX1 column (250 × 4.6 mm i.d., particle size 5μm), using mobile phase acetonitrile-water (3:97 *V/V)* at pH 3.0 adjusted with H_3PO_4. The flow rate was 2.0 ml/min. The detector wavelength was 215 nm, range 0.01 AUFS.

Reference solutions contain 0.28 - 0.42 mg/mL L-Lysinacetate in mobile phase, equivalent to 0.08 – 0.12 mg/mL ($1.36 \cdot 10^{-3}$- $2.04 \cdot 10^{-3}$ mol/L) acetate.

Test solutions are prepared by diluting the measured amounts of examined solutions with the mobile phase and filtering through 0.45 μm PTFE membrane filters.

A 20μL aliqout of each examined solution was injected in triplicate. All measurements were based on single injections. Since peak area measurements provided better perfomance than peak height, it was chosen for use in test procedure.

Acknowledgements

This work was supported by the Institute of Pharmacy of Serbia, Belgrade.

References and Notes

1. *Pharmacopeia Jugoslavica* **2000**, editio quinta, Savremena administracija, Beograd.
2. *U.S. Pharmacopeia 25, NF 20*, Board of Trustees **2002** 42, 855, 1574.
3. *British Pharmacopeia* **1998**. The Stationery office, London, Appendix VI A174.
4. *European Pharmacopoeia*, Fourth Edition **2002**, 576.
5. T. K. Natishan, and P. Sajonz, *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24*, 2583–2599.
6. T. Soga, M. Wakaura, *J. Am. Soc. Brew. Chem.* **1997,** 44-46.
7. Ionpac® ICE-AS1 ion exclusion column, *Dionex Corporation*, **1996**, Figure 1.

Povzetek

Za identifikacijo in določitev acetata v medicinskih produktih smo uporabili HPLC z anionsko izmenjevalno kolono in UV/VIS detektorjem. Na retenzijski čas vplivajo pH in ionska moč. Povečanje ionske moči na primer zmanjša retenzijski čas.