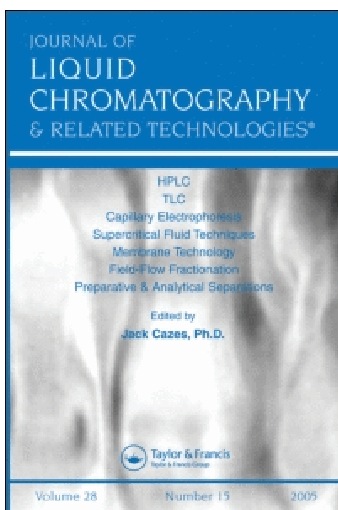
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

HPLC DETERMINATION OF 4-ACETYLAMINOPHENYLACETIC ACID

Fang Fang[a]; Guojie Xu[a]; Bing Lu[a]
[a] Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, P. R. China

Online publication date: 30 April 2001

To cite this Article Fang, Fang , Xu, Guojie and Lu, Bing(2001) 'HPLC DETERMINATION OF 4-ACETYLAMINOPHENYLACETIC ACID', Journal of Liquid Chromatography & Related Technologies, 24: 7, 1021 — 1027
To link to this Article: DOI: 10.1081/JLC-100103428
URL: http://dx.doi.org/10.1081/JLC-100103428

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 24(7), 1021–1027 (2001)

HPLC DETERMINATION OF 4-ACETYLAMINOPHENYLACETIC ACID

Fang Fang, Guojie Xu,* and Bing Lu

Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, 110015, P. R. China

Downloaded At: 09:32 24 January 2011

ABSTRACT

A fast and sensitive reverse phase ion-pair chromatographic method was reported for the separation and quantification of 4-aminophenylacetic acid (Actarit) and its related compounds. The compounds were separated using a Kromasil C18 column (5 µm, 4.6 × 25cm) with a mobile phase containing 70% methanol and 1% Tetrabutylammonium bromide in water flowing at 1.0 mL/min, and were detected using a UV detector operating at 245nm. Actarit was completely separated from its related compounds that included its synthetic starting materials and degradations. The retention times were 3.1, 4.0, 9.4, 21.4, and 25.8min for blank solvent, 4-aminophenylacetic acid, Actarit, 4-nitrophenylacetonitrile, and 4-nitrophenylacetic acid, respectively.

Three known and one unknown compound (retention time 13.7minute) were detected after Actarit was boiled in acidic, neutral, and basic conditions, each for two hours. The linear response between peak area (A) and Actarit concentration (c) over the range of 0.5 - 2.5 µg/mL was

$$A = -2285.20 + 40760.97c, r = 0.99993, (n = 5).$$

*Corresponding author. E-mail: juojie@uic.edu

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

The detection limit at signal-to-noise ratio two was 5.08 ng/mL. The recovery was 98.64 - 101.72% (n=5) (within day) and 99.60 - 101.86% (n=5) (day to day). The relative standard deviations were 0.87 - 1.77% (n=5) (within day) and 0.51 - 1.92% (n=5) (day-to-day), respectively. Good correlation was found between this HPLC method and a spectrophotometric method when measuring the contents of Actarit in three batches of commercial tablets. The contents were 97.32, 99.20, and 97.58% when measured by this HPLC method, and 97.76, 100.43, and 98.02% by a spectrophotometric method. Good separation, linearity, recovery, and accuracy implied that the chromatographic method is suitable for quantitative analysis of Actarit.

Downloaded At: 09:32 24 January 2011

INTRODUCTION

4-acetylaminophenylacetic acid (Actarit) is one kind of non-steroidal anti-inflammatory agent. In order to quantitatively analyze Actarit in dosage forms or in bio-samples, separations of Actarit from additives, from biological interferes and from degradation products of its own, are required. But to our knowledge, no study has been made on the analysis of Actarit by using HPLC. Only a radioactive (^{14}C-labeled) method has been reported previously[1].

Preliminary experiments showed that Actarit was always eluted out a regular C-18 column at dead time over a wide range of pH (2.5-9.5) and mobile phase polarity (methanol was increased from 0% to 100%). So a more complicated mobile phase is necessary to fulfill the chromatography separation. The purpose of this paper is to report a simple ion-pair chromatographic method to determine Actarit in tablet formulation.

EXPERIMENTAL

Materials

Tetrabutylammonium bromide, 4-aminophenylacetic acid (APA), and 4-nitrophenylacetic acid (NPA) were purchased from sigma (Sigma, USA). 4-nitrophenylacetonitrile (NPN) and 4-acetylaminophenylacetic acid (Actarit) were gifts from Eastern Greenland Inc. (Beijing, China).

Chromatographic Conditions and Instrumentation

The HPLC system consisted of a Waters 606 pump and Waters 486 UV detector (Waters, USA) operating at 245 nm. The injection loop was 10 μL.

The output signal from the detector was recorded and anlyzed by using Millenium soft system (Waters, USA) on a Dell computer. Separation was achieved using a Kromasil C18 column (5 µm, 4.6 × 25cm, Chaosheng corp, China). During analysis, 70% methanol-water solution containing 1% tetrabutylammonium bromide was used as mobile phase and flowed at 1.0 mL/min.

Preparation of Actarit Standard Solutions

A stock standard solution of Actarit was prepared in methanol at a concentration of 25 µg/mL. Working Actarit solutions ranging from 0.5 to 2.5 µg/mL were prepared by suitable dilution of the stock standard solution with the mobile phase. The standard solution of 0.5 µg/mL was further diluted to determine the limit of detection at the ratio of chromatography signal to noise of two.

Downloaded At: 09:32 24 January 2011

Preparation of Related Compound Solutions

The related compounds, 4-aminophenylacetic acid, 4- nitrophenylacetonitrile, and 4-nitrophenylacetic acid, were separately dissolved in methanol together with Actarit. The concentrations of Actarit and its related compounds were both 1 mg/mL.

Preparation of Hydrolyzed Actarit Sample Solutions

Actarit was dissolved in 0.1N hydrochloride, distilled water, and 0.1N sodium hydroxide, respectively. The final Actarit concentration was 2.5 µg/mL. Actarit solutions of 5 mL were sealed in 5 mL ampoules and were boiled for 2 hours, then the solutions were dried under vacuum; the residuals were dissolved in mobile phase to get the same concentration as its original.

Preparation of Sample Solutions from Tablets

An adequate quantity (equivalent to 50 mg of Actarit) of ground powder from 10 Actarit tablets were dispersed in 30 mL methanol in 50 mL volumetric flasks. After being supersonically vortexed for 5 minutes, the volumes were made up with methanol. The preparations were filtered through 0.45 µm membrane, and the filtrate was diluted suitably to obtain a concentration of about 2.0 µg/mL with mobile phase.

Sample Injection

Finally, 10 μL standard or sample solutions were injected into the HPLC system through the 10 μL injection loop.

RESULTS AND DISCUSSION

Chromatography

A typical chromatogram is shown in Figure 1. The retention times for blank solvent and Actarit were 3.1 and 9.4 min, respectively (Figure 1a). In order to test the system suitability, possible related compounds were also considered. The related compounds might be trace amount of 4-aminophenylacetic acid, 4-nitrophenylacetonitrile, and 4-nitrophenylacetic acid. They might come from a synthetic process or degradation. The retention time for 4-aminophenylacetic acid, 4-nitrophenylacetonitrile, and 4-nitrophenylacetic acid were 4.0, 21.4, and 25.8 minutes, respectively (Figure 1b-d). There are no apparent differences among the boiling samples of Actarit in acidic, neutral, and basic solutions.

All three known and one unknown related compounds (retention time 13.7 minute) were detected after the solutions were boiled for 2 hours. Figure 2 shows the chromatogram of boiled neutral Actarit solution. Good separation among Actarit and related compounds implied that the chromatographic conditions were suitable for quantitative analysis.

Downloaded At: 09:32 24 January 2011

Linearity, Precise, Recovery, and Limit of Detection

A linear response was established between peak area and Actarit concentration over the range 0.5 - 2.5 μg/mL. The regression equation was $A = -2255.20 + 40760.97c$ (A = area under peak, c = drug concentration in μg/mL). The correlation coefficient for linear regression was 0.99993 (n=5).

The recovery and accuracy were evaluated by adding known amounts of the drug to the control tablet formulation and were analyzed by the proposed method. The recovery was defined as the percentage of Actarit that was found after a known amount of standard was added to control formulation. The within day accuracy was determined by performing five repeats in eight hours. The day-to-day accuracy was determined by analyzing the same sample on three consecutive days.

Downloaded At: 09:32 24 January 2011

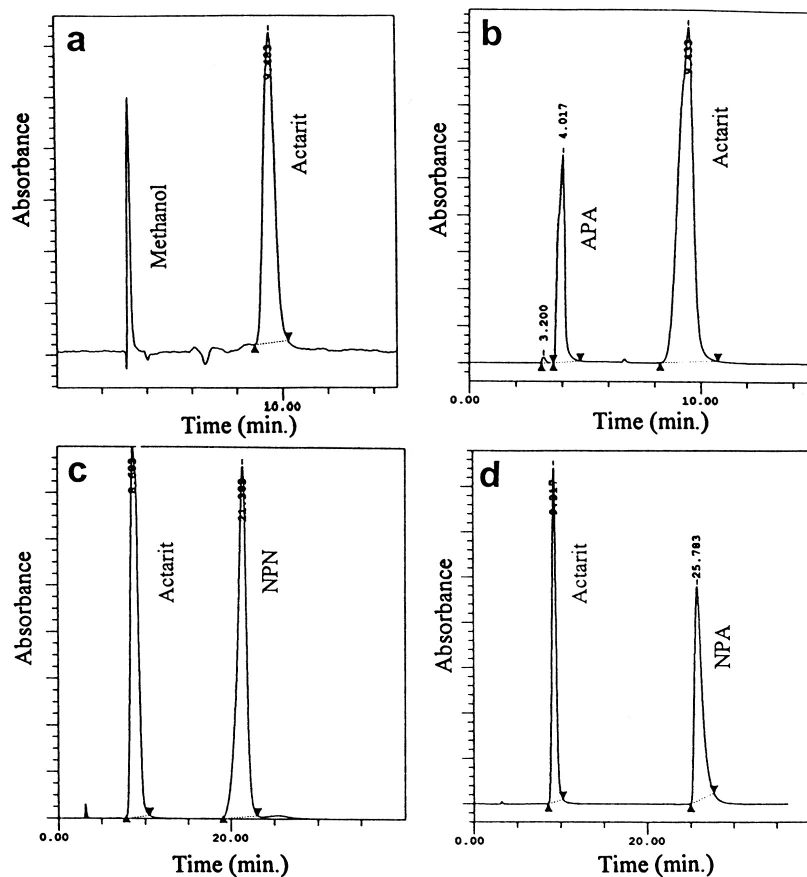


Figure 1. Typical chromatograms of Actarit and its related compounds on a Kromasil C18 column using a 70% methanol-water solution containing 1% tetrabutylammonium bromide at 1.0 mL/min flow rate and detection at 245 nm (Solvent 3.1min; APA, 4.0min; Actarit, 9.4min; NPN, 21.4min; and NPA, 25.8min).

The recovery data obtained from this study was 98.64% - 101.72 (n=5) (within day) and 99.60% - 101.86 (n=5) (day to day). The relative standard deviations were 0.87 –1.77% (n=5) (within day) and 0.51 – 1.92% (n=5) (day-to-day), respectively (Table 1).

The detection limit, which was defined as the minimum quantifiable amount of Actarit at signal-to-noise ratio of two, was 5.08 ng/mL.

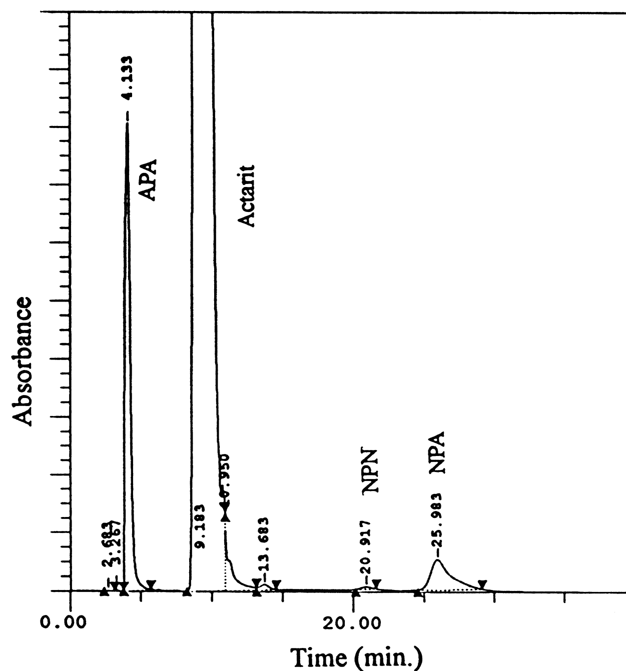


Figure 2. Chromatogram of Actarit solution after boiled for 2 hours (APA, 4.1min; Actarit, 9.2min; NPN, 20.9min; and NPA, 26.0min).

Downloaded At: 09:32 24 January 2011

Practical Application

This HPLC method was used in analyzing the Actarit in three batches of commercial tablets. The measured contents were 97.32 ± 1.71, 99.20 ± 0.88 and 97.58 ± 1.03% (n=3) of labeled contents, respectively. These results were very

Table 1. The Recovery and Accuracy of Actarit Determinations

	Amount Added ($\mu g/mL$)	Amount Found ±SD ($\mu g/mL$)	Accuracy (RSD, %)	Recovery %
	0.508	0.501±0.009	1.77	98.64
Within-day	1.525	1.551±0.024	1.54	101.72
	2.542	2.560±0.022	0.87	100.73
	0.508	0.506±0.003	0.59	99.60
Day-to-day	1.525	1.553±0.030	1.92	101.86
	2.542	2.552±0.013	0.51	100.40

close to the contents measured using a spectrophotometric method, which were 97.76 ± 0.81, 100.43 ± 1.03, 98.02 ± 0.99% (n=3), respectively.

ACKNOWLEDGMENT

The current address for Fang Fang and Guojie Xu is: Department of Pharmaceutics and Pharmacodynamics (M/C 865), College of Pharmacy, University of Illinois at Chicago. 833 South Wood Street, Chicago, IL 60612.

REFERENCE

1. Sugihara, K.; Morino, A.; Nomura, A.; Iida, S.; Sugiyama, M. Pharmacokinetics of 4-Acetylaminophenylacetic Acid. 1st Communication: Absorption, Distribution, Metabolism and Excretion in Mice, Rats, Dogs and Monkeys After Administration of 14C-Labeled Compound. Arzneimittelforschung **1990**, *40* (7), 800–805.

Received January 24, 2000 Manuscript 5251
Accepted October 18, 2000

Downloaded At: 09:32 24 January 2011