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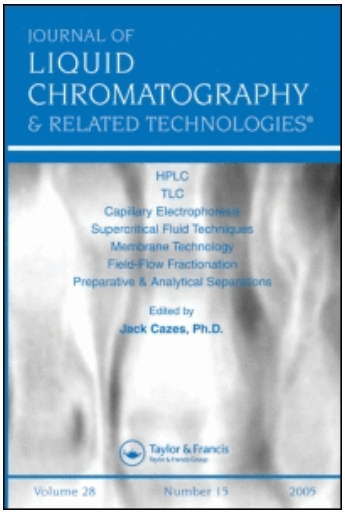

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Analysis of Acetaminophen and Salicylate by Reverse Phase HPLC

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ANALYSIS OF ACETAMINOPHEN AND SALICYLATE BY REVERSE PHASE HPLC

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ABSTRACT

A method for the simultaneous measurement of acetaminophen and salicylate by reverse phase HPLC using N,N-Diethyl-m-toluamide as internal standard is described. The method, which utilizes a one step acetonitrile precipitation of serum, can be applied to as little as 25μL of sample. Serum is Vortex-mixed with acetonitrile containing the internal standard and following centrifugation an aliquot of the supernatant is analyzed on a reverse-phase column. The eluting solvent consists of methanol/5% acetic acid (60/40,V/V) and the effluent is monitored at 248 nm.

INTRODUCTION

Both acetaminophen and salicylate are used as analgesics and antipyretics and both are generally safe. However, hepatic necrosis or fulminant hepatic failure can result from acute or severe overdose (1-3). The widespread availability and use of these drugs is a frequent cause of accidental poisoning in children. Since the time for hepatic injury to become biochemically apparent requires from 1 to 2 days (4,5) it is useful to monitor the serum levels over a period of time.

There have been time consuming gas-liquid (6) and spectrophotometric methods (7,8) described as well as several liquid chromatographic procedures (9,10,11). However, the HPLC methods either employ no internal standard, consist of a multistep extraction procedure, or do not determine both drugs simultaneously.

In this paper an HPLC method for the simultaneous determination of acetaminophen and salicylate is described, in which N,N-Diethyl-m-toluamide

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is used as an internal standard. The analysis is fast and has been successfully applied to patient specimens in our pediatric servive laboratory. By adjusting the detector to 0.01 absorbance units full scale (AUFS) we have been able to measure as little as 1 µg/ml of each drug.

MATERIALS AND METHODS

Instrumentation

A constant volume liquid chromatograph from Waters (Milford, MA.) consisting of a model 6000A solvent delivery system, a U6K sample injector and a model 450 detector (interfaced with a Waters 730 Data System) set at 248 nm was used. The detector was set at 0.1 AUFS for routine analysis. The column was a prepacked µ Bondapak C18 also from Waters.

Procedure

Add 200 µL of acetonitrile, containing 80 mg/L of IS, to 50 µL of serum in a 10x75 mm glass test tube. Vortex-mix for 10 sec., followed by a 2 min. centrifiguation (2500 r.p.m.). An aliquot of the supernatant (usually 20 µL) was then injected into the chromatograph. We observed no differences when 25 µL of serum and 100 µL of acetonitrile were used. When using this smaller size sample we found it more convenient to use an 0.3 ml size Mini-Vial (The Aspect Co., Inc., Ann Arbor, MI).

We quantified samples with the Waters 730 Data System using the peak height calibration mode. We found that the peak height calibration mode gave more accurate reproducibility for off scale peaks than did the peak area calibration mode.

For daily routine analysis of patient samples we use two levels of serum controls, prepared by adding acetaminophen and salicylate to drug free serum. One of these, containing 30 mg/L acetaminophen and 100 mg/L salicylate, is routinely used for the Data Module internal standard calibration; and the other, containing 20 mg/L acetaminophen and 69 mg/L salicylate, is used as the procedure control.

RESULTS AND DISCUSSION

Some typical HPLC chromatograms obtained using our procedure are shown in Figure 1. The retention times for acetaminophen, salicylate and the internal standard were 2.49, 3.84 and 5.35 min. respectively. These retention times have never varied more than 0.03 min. Figure 1A is a chromatogram of a drug free serum. Figure 1B is our control serum containing 30 mg/L acetaminophen (A) and 100 mg/L salicylate (S). Figure 1C is a patient sample calculated to contain no acetaminophen and 50 mg/L salicylate.

Recovery

Recoveries were assessed by comparing data obtained from a 50 µL aliquot of a methanol solution containing 30 mg/L acetaminophen and 100 mg/L

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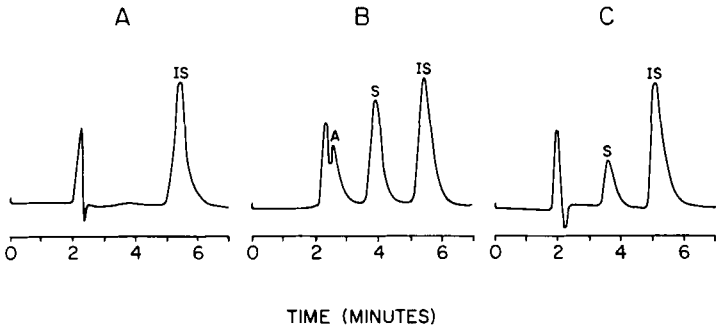

Figure 1. Typical chromatograms, obtained using the procedure given herein. Retention times: acetaminophen, 2149 min.; salicylate, 3.84 min; internal standard, 5.35 min. (A) drug-free serum; (B) control serum containing 30 mg/L acetominophen and 100mg/L salicylate; (C) patient sample, calculated to contain no acetominophen and 50 mg/L salicylate.

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salicylate to which 200 µL of the acetonitrile-IS was added, and our serum control containing the same levels of each drug. We obtained recoveries from 96.3 to 101.2%, including the internal standard.

We also compared these recoveries to those obtained using two different extraction procedures (9,10). Using chloroform/isopropanol (9) we obtained 95 to 97% of our procedures recovery and using ethyl acetate (10) the values were less, yielding 85 to 89% of our values.

Precision

Day to day precision was assessed by assaying our control samples over a week period. The overall average of the mean coefficient of variation was 4.83% for the acetaminophen and 4.68% for salicylate. The within-run precision was evaluated by applying the method 10 times to a control serum. The mean coefficient of variation was 4.42% and 4.31% for acetaminophen and salicylate respectively.

Linearity

Acetaminophen and salicylate were added in varying concentrations to drug free serum and the samples analyzed. The results showed the present method to be linear over the ranges we tested, which were 10 mg/L to 300 mg/L for acetaminophen and 25 mg/L to 700 mg/L for salicylate.

Some typical results from a duplicate assay of various commercial serum controls are given in Table I. As can be seen all values are within the expected range. A duplicate of the control sample from our laboratory had been used to calibrate the Data Module.

The assessed accuracy, high reliability and simplicity makes the present method easily applicable to a clinical laboratory. The sample size requirements make this an especially useful method to a pediatric service laboratory.

TABLE 1

Results Of Assaying Various Controls

	Acetaminophen, mg/L		Salicylate, mg/L	
Sample	Expected	Found	Expected	Found
Sera Chem[1]	0	0	170-230	187.4
	0	0.69		183.6
Ortho I[1]	6-14	12.3	180-270	223.4
		11.9		220.6
Ortho II[2]	26-38	38.4	450-550	536.2
		35.5		534.3
Control[3]	20	19.2	69	70.3
		20.2		66.3

[1]Fisher Scientific Co., Orangebury, NJ

[2]Ortho Diagnostics, Raritan, NJ

[3]Our control made from drug free serum

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