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Note

Rapid high-performance liquid chromatographic determination of acetaminophen in dosage form using a totally aqueous mobile phase

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Over the last decade, acetaminophen has become a widely used substitute for aspirin and aspirin-type analogues. Since it contains no carboxylic acid functionality, it is promoted as an effective analgesic and antipyretic with gentler gastrointestinal properties. As a result of its rapid success and popularity as an alternative medication, the analysis of acetaminophen has received considerable attention.

In just the last half dozen years, numerous techniques have been employed for the analysis of acetaminophen in biological samples. Various chromatographic[1–3], spectrophotometric[4], and electrochemical[5] methods have been reported. Of the liquid chromatographic procedures, reversed-phase separations employing an octadecyl column often are utilized[6,7].

The quantitation of acetaminophen in various dosage forms also has received considerable attention. Titration with tetraalkylammonium hydroxides[8,9], colorimetric methods[10–12] and an oxidative electrochemical reaction using a carbon electrode[13] have been reported. Gas and liquid chromatographic[14,15] assays for acetaminophen in dosage forms also have been devised.

Recently published high-performance liquid chromatographic (HPLC) methods have employed both silica and alkyl phases as packings. Many of these separations require moderately long analysis times. For example, the use of a silica column and a mobile phase consisting of butyl chloride, tetrahydrofuran, methanol and glacial acetic acid has been reported to require *ca.* 30 min per sample[15].

The current HPLC procedure is a rapid simple method for the analysis of acetaminophen in tablets and capsules either as single or composite samples. It is designed as an internal standard method for process control. The procedure consists of a simple dissolution step followed by a single dilution. After sample preparation, the complete chromatographic analysis takes *ca.* 2 min per sample injection, using only water as the mobile phase.

EXPERIMENTAL

Reagents

All separations were carried out on 5 cm × 4.5 mm I.D. octyl columns obtained from IBM Instruments (Danbury, CT, U.S.A.). Prior to use, the column was phase-rearranged by initially conditioning with at least 50 ml of acetonitrile followed

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by an equivalent volume of water. The flow was turned off for an additional 20 min. This conditioning was carried out at 55°C. The column was then brought to thermal equilibrium at the analysis temperature of 40°C.

The acetonitrile was distilled-in-glass UV grade obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). All water was obtained from a Barnstead (Boston, MA, U.S.A.) 4-module NANO pure purification system.

Equipment

Column temperature was controlled to 0.1°C using a Neslab (Portsmouth, NH, U.S.A.) Model EX-300 temperature and Model DCR-1 digital controller. All detection was at 254 nm.

Procedure

A 0.5 mg/ml internal standard solution was prepared by dissolving 50 mg of methyl *p*-hydroxybenzoate in 100 ml of water. Calibration curve standards were prepared by quantitatively adding respectively 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of 1.30 mg/ml acetaminophen standard to 10-ml volumetric flasks. All flasks were volumetrically filled following the addition of 2.0 ml of internal standard solution to each.

Individual tablet, capsule or equivalent composite samples were placed in 250-ml volumetric flasks to which *ca.* 100 ml of water were added. The mixtures were shaken for 20–30 min after which the content of each flask was diluted to volume. After allowing a solution to settle for at least 15 min, 0.5 ml of it was transferred to a 10-ml volumetric flask. Then 2 ml of internal standard were added and the contents were diluted to volume with water.

Aliquots of 5 μl of the above samples and standards were chromatographed on a 5-cm octyl column using 100% water as the mobile phase. In all cases, the flow-rate was maintained at 2.0 ml/min at a column temperature of 40°C.

RESULTS AND DISCUSSION

Shown in Fig. 1 are representative chromatograms of acetaminophen and the internal standard methyl *p*-hydroxybenzoate. These data were obtained from a series of calibration standards. All separations were carried out, using a totally aqueous mobile phase, on a 5-cm octyl column in the phase-rearranged orientation.

The concept of phase rearrangement has been discussed previously[16,17]. Briefly, surfaces with chemically attached hydrocarbon moieties of appropriate chain length and silane-backbone structures can be oriented in one of two configurations in a totally aqueous environment. These two arrangements have been termed the folded or down-state and the extended or up-state. The down-state is obtained after an initial conditioning procedure with an organic water-miscible solvent, usually methanol or acetonitrile, followed by treatment with water. The up-state is formed by thermal rearrangement at elevated temperatures. For the particular surface used, treatment at 55°C was sufficient for formation of the up-state configuration.

As the result of phase rearrangement, solutes may be made to elute rapidly or to be retained for greater lengths of time, depending on the nature of the bonded alkyl chains. Shorter retentions are obtained when the phase is wetted or in the up-state

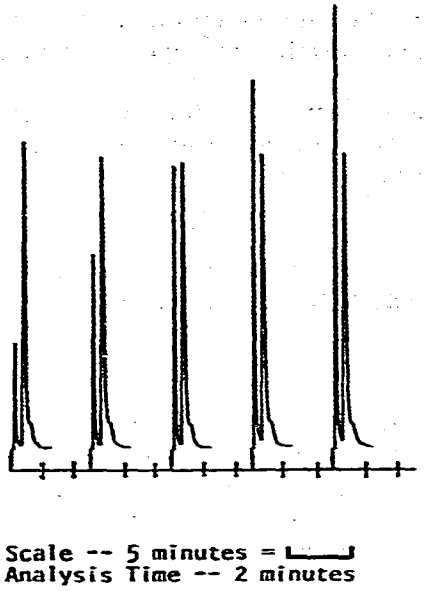

Fig. 1. Representative chromatograms of acetaminophen standard solutions. Peaks: first peak, acetaminophen; second peak; methyl *p*-hydroxybenzoate. Conditions: mobile phase, 100% water; flow-rate, 2.0 ml/min; column temperature, 40°C.

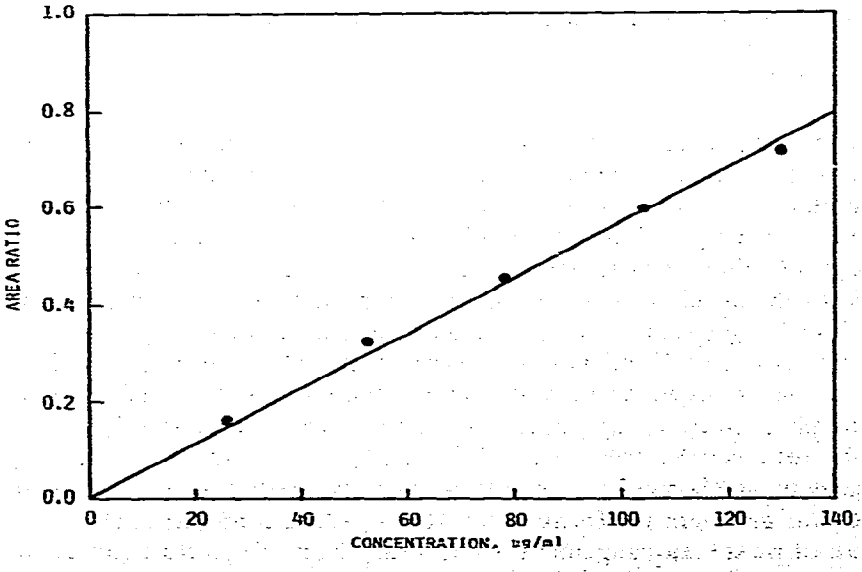

Fig. 2. Calibration curve for the area ratio of acetaminophen to internal standard (*i.e.*, methyl *p*-hydroxybenzoate) *vs.* concentration of acetaminophen in µg/ml.

orientation. This arrangement greatly reduces retentivity of the surface, permitting solutes which would ordinarily be retained for relatively long periods of time in a totally aqueous mobile phase to be analyzed more rapidly. In some cases, changes in retention of almost two orders of magnitude have been produced[18].

Shown in Fig. 2 is a plot of area ratio of acetaminophen to internal standard *vs.* acetaminophen concentration. The plotted points represent the means of at least five replicate injections per standard. The plot showed good linearity over the desired concentration range (*i.e.*, up to 130 μg/ml).

Summarized in Tables I and II are results from single 325-mg tablet and composite analyses. These values represent the mean of at least five replicate injections per sample. The overall single tablet variation was from 98.6% to 102.4% acetaminophen with an average value of 100.2% for the ten trials. This is in excellent agreement with an overall average value for the composite samples of 99.8% and a variation of 98.7% to 101.4% for the individual trials.

Summarized in Table III are the analysis results from single 500-mg capsules. The precision of these data is in good agreement with that obtained on the single

TABLE I

SINGLE 325-mg ACETAMINOPHEN TABLET RESULTS

Sample	*Percentage**
Tablet 1	100.1
Tablet 2	99.4
Tablet 3	101.3
Tablet 4	99.2
Tablet 5	98.6
Tablet 6	98.8
Tablet 7	100.1
Tablet 8	100.6
Tablet 9	102.4
Tablet 10	101.7
Average value	100.2 ± 1.3%

* Mean of at least five replicate injections per sample.

TABLE II

COMPOSITE 325-mg ACETAMINOPHEN RESULTS

Sample	*Percentage**
Weighing 1	99.2
Weighing 2	99.4
Weighing 3	101.4
Weighing 4	98.7
Weighing 5	100.2
Average value	99.8 ± 1.1%

* Mean of at least five replicate injections per sample.

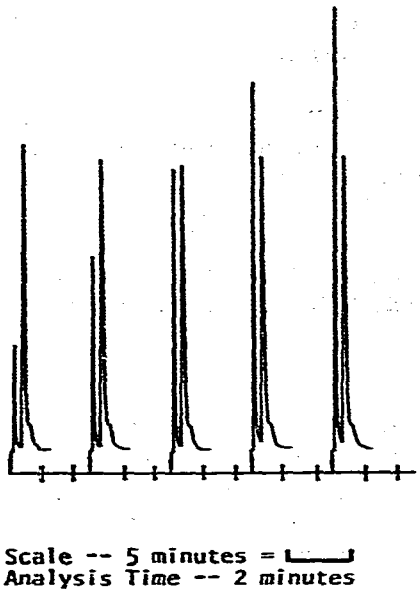

Fig. 1. Representative chromatograms of acetaminophen standard solutions. Peaks: first peak, acetaminophen; second peak; methyl *p*-hydroxybenzoate. Conditions: mobile phase, 100% water; flow-rate, 2.0 ml/min; column temperature, 40°C.

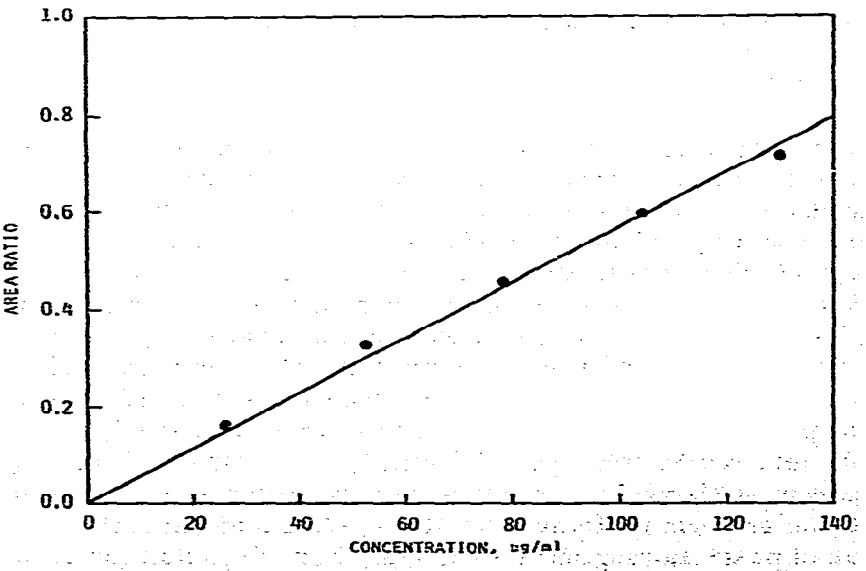

Fig. 2. Calibration curve for the area ratio of acetaminophen to internal standard (*i.e.*, methyl *p*-hydroxybenzoate) *vs.* concentration of acetaminophen in µg/ml.