Stability-Indicating Assay of Acetaminophen in an Effervescent Tablet by Ion-Pair High-Performance Liquid Chromatography

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Abstract □ A stability-indicating analytical method is described for determining acetaminophen and its degradation product, *p*-aminophenol, in an effervescent tablet. Tablets assayed by ion-pair high-performance reversed-phase liquid partition chromatography required no sample cleanup. The method applied to tablets containing 325 mg of acetaminophen yielded an average recovery of 99.6% with a relative standard deviation of 0.70% (n = 10). As little as 0.005% *p*-aminophenol could be detected.

Keyphrases □ Acetaminophen—analysis, ion-pair high-performance liquid chromatography, effervescent tablets, with *p*-aminophenol, stability indicating □ *p*-Aminophenol—analysis, ion-pair high-performance liquid chromatography, effervescent tablets, with acetaminophen, stability □ Analgesics—acetaminophen, ion-pair high-performance liquid chromatographic analysis, effervescent tablets, with *p*-aminophenol, stability □ High-performance liquid chromatography—analysis, acetaminophen, *p*-aminophenol, effervescent tablets

Acetaminophen is used extensively in nonprescription analgesic preparations. The current USP assay (1) for acetaminophen and its degradation product, *p*-aminophenol, in tablets, while selective and sensitive, is time consuming and tedious. Additionally, the novel matrix of an effervescent tablet presents interferences not commonly encountered in the assay for *p*-aminophenol, rendering it nonapplicable as a stability-indicating assay. Other rapid methods for acetaminophen involve either the formation of *O*-heptyl-*N*-methyl derivatives followed by GLC (2) or hydrolysis to *p*-aminophenol, followed by oxidation to *p*-quinonechlorimide and reaction with phenol to form indophenol, which is then determined colorimetrically (3). These methods assay the total drug content and are not suitable for simple one-step differentiation between intact acetaminophen and its degradation products. Other

Table I—Recovery Percentage for Spiked Samples Containing 325 mg of Acetaminophen

Sample	Recovery of Acetaminophen, %
1	102.6
2	102.4
3	100.9
4	99.9
5	100.1
6	99.7
7	101.6
8	99.3
Average	100.8
RSD, %	1.28

Table II—Assay Results for Effervescent Tablets Containing 325 mg of Acetaminophen

Sample	Acetaminophen Found, mg	Percent of Label
A	322.7	99.3
B	323.4	99.5
C	322.1	99.1
D	322.7	99.5
E	322.4	99.2
F	328.0	100.9
G	326.7	100.5
H	322.7	99.5
I	321.4	98.9
J	326.0	100.3
Average	323.8	99.6
RSD, %	0.70	0.70

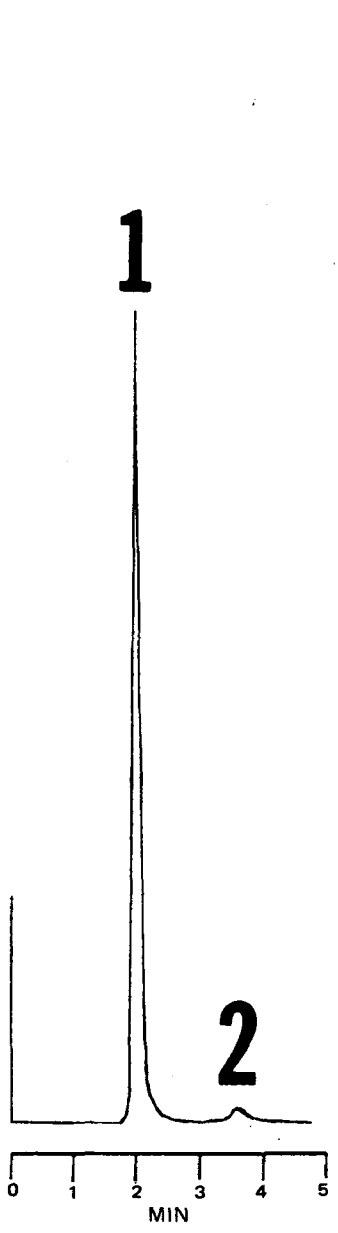

Figure 1—*Representative chromatograms. Key: 1, acetaminophen; and 2, tablet excipient.*

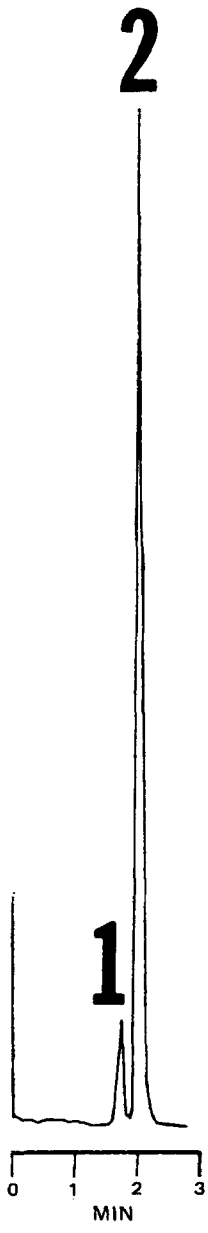

Figure 2—*Separation of mixture containing acetaminophen and* p*-aminophenol. Key: 1,* p*-aminophenol; and 2, acetaminophen.*

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chromatographic methods for intact acetaminophen and *p*-aminophenol are too lengthy for large numbers of samples (4, 5) or require unusual apparatus for measuring trace amounts of *p*-aminophenol (6).

To overcome these problems, ion-pair high-pressure partition liquid chromatography was investigated. This method was simple, rapid, precise, and selective for determining the acetaminophen concentration in an effervescent tablet matrix while providing a measurement of the *p*-aminophenol present.

EXPERIMENTAL

Chemicals and Reagents—Acetaminophen USP[1] was assayed spectrophotometrically by comparison to the USP reference standard and was used without further purification. *p*-Aminophenol[1] (98+%), acetonitrile[2] (UV, distilled in glass), and tetrabutylammonium phosphate solution[3] were used as received.

Apparatus—The liquid chromatograph[4] was fitted with a septumless injector[5], a fixed-wavelength UV detector[6] (254 nm), and a strip-chart recorder.

Column—A 30-cm × 4-mm i.d. column containing 10-μm phenyl bonded phase packing[7] was used.

Chromatographic Conditions—The chromatographic solvent was 0.005 *M* tetrabutylammonium phosphate in distilled water containing 15% (v/v) acetonitrile, adjusted to pH 7.5 with phosphoric acid or sodium hydroxide. The solvent was vacuum filtered through a 0.45-μm filter[8] and vacuum degassed for 2 min with stirring before use. The temperature was ambient, the solvent flow rate was 3.0 ml/min, and the inlet pressure was 2000 psig. Sensitivities used were 0.2 aufs for acetaminophen and 0.005 aufs for *p*-aminophenol.

Standard Solutions—Standard solutions containing 1.0, 1.4, 1.8, and 3.0 mg of acetaminophen/100 ml were prepared in distilled water containing 15% (v/v) acetonitrile and filtered as described prior to injection.

Assay for Pharmaceuticals—Each tablet containing 325 mg of acetaminophen was dissolved in 100.0 ml of distilled water containing 15% (v/v) acetonitrile. One milliliter of this solution was pipetted into a 200-ml volumetric flask, diluted to volume with the same solvent, and filtered as described before injection.

Spiked Samples—Accurately weighed quantities of acetaminophen were admixed with a placebo granulation of the effervescent tablet. These mixes, formulated to contain 325 mg/tablet weight (the same percentage of acetaminophen as found in the tablets), were assayed as described.

Quantitation—Since peak heights of acetaminophen were directly proportional to concentration, all results were calculated using peak heights.

RESULTS AND DISCUSSION

The recovery data for acetaminophen from spiked samples presented in Table I illustrate the validity of the method for effervescent tablets. Average recovery for spiked samples was 100.8%. Linearity between peak height and concentration was excellent over the range of 1.0–3.0 mg of acetaminophen/100 ml and had a correlation coefficient of 0.9998. This method has been used routinely in a stability program and found to be accurate and precise with a relative standard deviation of 0.70% ($n = 10$). These data are summarized in Table II. A chromatogram obtained from a typical tablet assay is presented in Fig. 1. Since no sample cleanup is required, the assay time is only 20 min compared to 3–4 hr by the official USP procedure.

To demonstrate the selectivity of the method for acetaminophen in the presence of *p*-aminophenol, samples of acetaminophen containing 10, 1, 0.1, and 0.005% *p*-aminophenol were prepared and assayed. A chromatogram of acetaminophen containing 10% *p*-aminophenol is given in Fig. 2. Excellent resolution of *p*-aminophenol from its parent compound, an essential requirement for a stability-indicating assay, was obtained. Quantification of 0.005% *p*-aminophenol required the optimization of assay conditions, namely, maximization of both the acetaminophen sample size and the UV detector sensitivity. Average recovery at the 0.005% *p*-aminophenol level was 96.9% with a standard deviation of ±4.55 ($n = 4$). This separation could not be achieved with a reversed-phase C_{18} column using the same chromatographic conditions. Presumably, the π-bond to π-bond interaction between the phenyl stationary phase and the phenyl backbone of the solute molecules provides the necessary selectivity for the separation of acetaminophen and *p*-aminophenol.

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[1] Mallinckrodt Chemical Co.
[2] Burdick & Jackson Laboratories.
[3] PIC reagent A, Waters Associates.
[4] Model 6000A pump, Waters Associates.
[5] Valco model CV-6-UHPa-N60, 7000-psig sample injection valve equipped with a 10-μl sampling loop.
[6] Model 440, Waters Associates.
[7] μBondapak Phenyl, Waters Associates.
[8] HA filters, Millipore Corp.