quality control use, and the results compare favorably with the current official procedure.

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Detection Limits for a GC Determination of Methanol and Methylene Chloride Residues on Film-Coated Tablets

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Abstract □ A GC assay was developed that quantitates methanol and methylene chloride at the lowest detectable levels for this mode of analysis. A statistical limit of detection was determined for both methanol and methylene chloride. This method is sensitive and reliable for detecting possible residues of these solvents on film-coated tablets.

Keyphrases □ Methanol—determination of detection limits for a GC method, methylene chloride, residues on film-coated tablets □ Methylene chloride—determination of detection limits for a GC method, methanol, residues on film-coated tablets □ Detection limits—determination, GC method for methanol and methylene chloride residues on film-coated tablets □ Film-coated tablets—determination of detection limits for a GC method, methanol and methylene chloride residues

Organic solvents are frequently used to dissolve film-coating materials such as methylcellulose and ethylcellulose to facilitate application onto compressed tablets. These tablets are subsequently air dried for varying periods of time until constant tablet weight is achieved. The assumption was made that drying removes all the organic solvents from the finished product. However, there are a scarcity of data documenting the actual organic solvent levels that may be found in the coated tablet.

Patt and Hartmann (1) studied the effect of tablet core porosity, spraying techniques, drying condition, and evaporation qualities of the solvents used during the film-coating process. The residual levels of organic solvents in the tablet cores and film coats were determined by a GC method. In the present study, a similar GC system was used to determine the levels of methanol and methylene chloride in tablets at various times during the film-coating process. These levels were monitored during a 24-h period of air drying after the film coating was complete. The analytical data were then evaluated by a method similar to Hubaux and Vos (2) to determine a statistically based limit of detection for this method. This technique is similar to the statistical methods defined by Parsons (3) and Currie (4).

EXPERIMENTAL

Materials—A GC[1] equipped with a flame-ionization detector was used with a 1.8-m × 3-mm coiled-glass column containing 80–100 mesh porous polymer packing[2]. Reagents included isopropyl alcohol (AR), chloroform, methanol, and methylene chloride; all reagents were glass distilled[3].

Tablet Coating—A solution of ethylcellulose and methylcellulose in methanol and methylene chloride was sprayed[4] onto the compressed tablets in a heated, rotating coating pan[5]. Uniform coating was achieved when a coating of ~3% of the tablet weight was deposited.

Analytical Procedure—Standard stock solutions of methanol and methylene chloride at concentrations of 24 and 40 ppm were prepared in chloroform, and dilutions were injected onto the GC at the lowest attenuation (where the baseline noise did not exceed 2 mm). The volume of injection of these dilutions was 2.5 μL. Quantitation of the peaks was done with isopropyl alcohol as the internal standard. At an oven temperature of 160°C and a gas flow rate of 60 mL/min, the methanol eluted at ~1–2 min, the methylene chloride at 3–4.5 min, and the isopropyl alcohol at 4–5 min. The limit of detectability, as determined by the instrument at a slope sensitivity of 1 and an attenuation of 8, was ~6 ppm for methanol and 10 ppm for methylene chloride. A calibration curve was prepared by injecting a series of six dilutions which varied fourfold in concentration. The range for methanol was 6–24 ppm, and for methylene chloride the range was 10–40 ppm.

A series of six spiked placebos at concentrations of 0–24 ppm for methanol and 0–40 ppm for methylene chloride were prepared to determine the linearity and recovery over the range of interest. A placebo was defined as a tablet manufactured with no organic solvents in the film-coating formulation.

Coating Studies—Once the lower limit was approximated and a range of linearity established, an experiment was designed to detect methanol and methylene chloride on tablets during the film-coating process. The film-coating formulation consisted of the following ingredients: hy-

Table I—Residual Methanol and Methylene Chloride During and After Film Coating

Sample	Concentration, ppm per tablet[a] Methanol	Methylene Chloride
Uncoated tablet (control)	0	0
5-min coating process	173	52
10-min coating process	312	74
15-min coating process	252	66
Immediately after coating	242	55
5-min heat drying	143	37
15-min air drying	118	31
30-min air drying	115	30
24-h air drying	—[b]	—[b]

[a] Average tablet weight is 760 mg. [b] — No detectable levels.

[1] Hewlett-Packard Model 5830A.

[2] Poropak R packing; Waters Associates, Milford, Mass.

[3] Glass-distilled solvents; Burdick and Jackson, Muskegon, Mich.

[4] Spray gun apparatus; Devilbiss, Toledo, Ohio.

[5] Erweka AR 400 coating pan.

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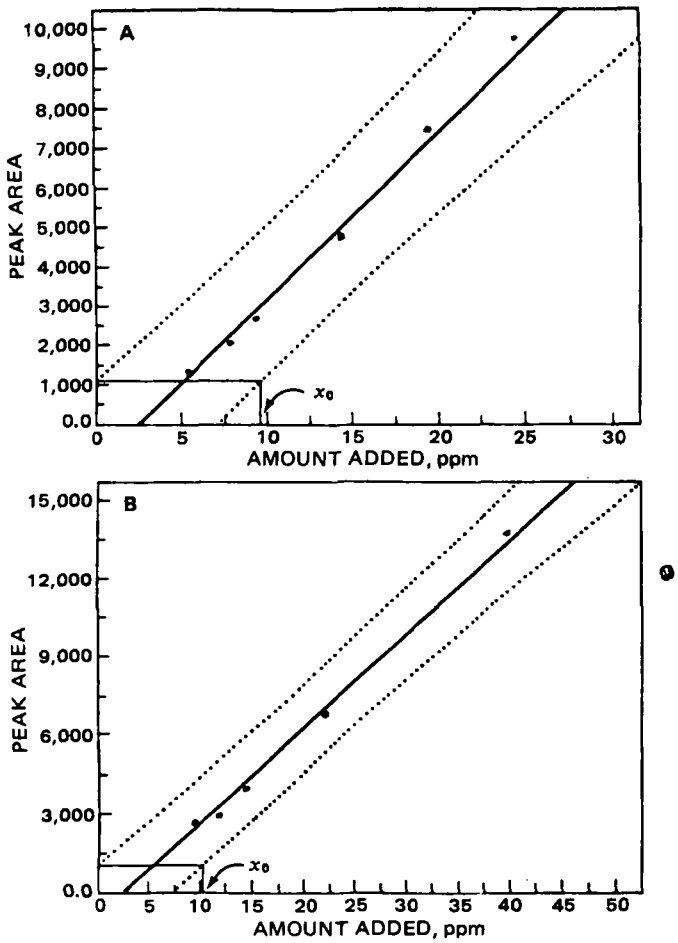

Figure 1—*Limit of detection curves for methanol (A) and methylene chloride (B) showing the statistical lower limit of detection as defined by Strobel (5). The lower limits of detection (x_0) were 9.552 ppm for methanol and 10.16 ppm for methylene chloride. Key: (—) regression line; (. . .) upper and lower 95% confidence lines.*

droxypropyl methylcellulose, ethylcellulose[6], diethyl phthalate, methanol, and methylene chloride. The film-coating process was performed using a coating pan and spray gun apparatus on a currently marketed tablet. Sampling consisted of pulling five tablets at each time interval of the coating process. Immediately after sampling, the tablets were submerged in 50 mL of chloroform to ensure complete recovery of the solvent residues. Tablets were collected from the coating pan after 5, 10, and 15 min of coating and immediately after the pan stopped. Additional collections were made after 5 min of heat drying and after 15 and 30 min of air drying; a sample was also taken after 24 h of air drying. The tablets were stored in plastic bags until packaging. Coated tablets from previous batches which had been packaged and maintained at room temperature were also assayed. Uncoated tablets were assayed as a control.

RESULTS AND DISCUSSION

Figure 1 represents the calibration curves used to statistically determine the lowest detectable limit with 95% confidence (2, 5). Concentration was plotted against peak area ratio, and a regression line was fitted through these points with confidence intervals[7]. The point of interception of the upper confidence line at the y-axis was extended horizontally to the lower confidence line, and where the two lines intersected a perpendicular was dropped to the x-axis. Parsons defines this point as the detection limit (3). For methanol, the value of this point was ~9 ppm per average tablet weight (Fig. 1). For methylene chloride, the value was approximated at 10 ppm per tablet (Fig. 1). Figure 2 shows the detector response for these values.

The results of the spiked placebo evaluation curve, evaluating solvent

[6] Methocel E-15 Premium, Ethocel 10 cps; Dow Chemical Co., Midland, Mich.
[7] Hewlett-Packard HP 85 Desk Top Computer equipped with an in-house program written for limits of detection determinations.

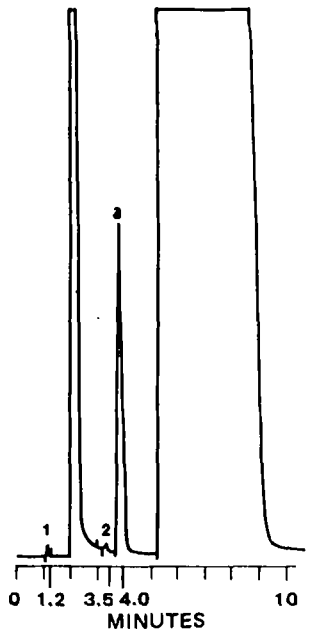

Figure 2—*Chromatogram showing detector response at the detection limit of methanol (1) and methylene chloride (2), with internal standard (3).*

added against the amount of solvent found, proved to be linear over the range studied. The precision of the method was determined by evaluating the results of six replicate injections of each standard solution. The standard deviations of the peak area measurements were 3.68% for methanol and 4.22% for methylene chloride. All samples and spiked placebos were evaluated against a standard containing 24 ppm of methanol and 40 ppm of methylene chloride (Fig. 3). Isopropyl alcohol was chosen as an internal standard because it was well resolved from the other peaks.

The results of the film-coating procedure are seen in Table I. Figure 4 depicts the methanol and methylene chloride peaks after 15 min of coating, where the residues are at a maximum. Table I shows that 24 h after the coating process, before packaging, no solvent residues were detected. No residues of methanol or methylene chloride were detected from the control or the older coated tablets.

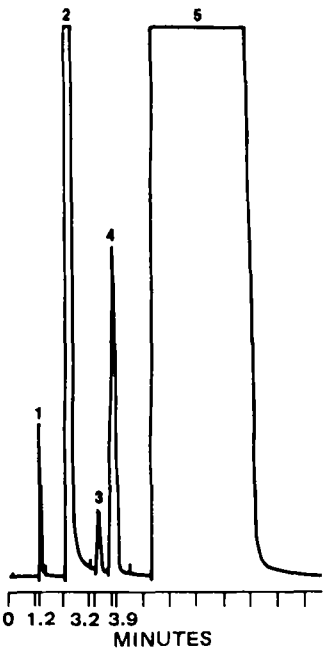

Figure 3—*Standard chromatogram for methanol and methylene chloride. Key: (1) methanol; (2) ethanol from solvent; (3) methylene chloride; (4) isopropyl alcohol (internal standard); (5) chloroform-solvent peak.*

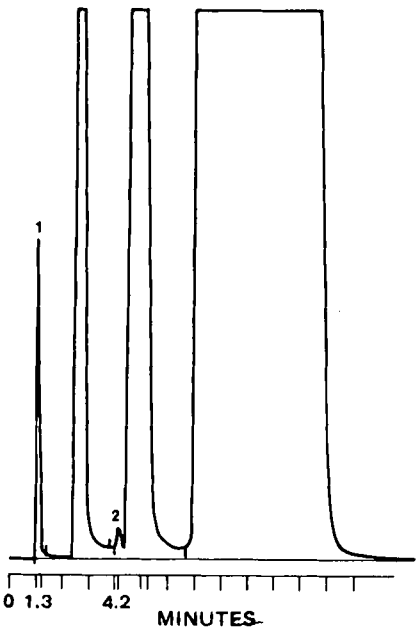

Figure 4—*Chromatogram of freshly coated tablets before drying, showing both methanol and methylene chloride after 15 min of coating. Key: (1) methanol; (2) methylene chloride.*

Statistical limits of detection were established for methanol at 9.5 ± 1.5 ppm per average tablet weight and for methylene chloride at 10.1 ± 2.3 ppm per tablet. Below these limits, sensitivity of the instrument was unreliable. All absolute values were approximate due to two factors. First, precision of measurement is decreased as concentrations used in the calibration curve approach the detection limit. As an alternative, a calibration curve could have been drawn at higher concentrations and extrapolated to the limit of detection (3). Second, this detection limit is also dependent on where the confidence lines are drawn about the regression line and is, at best, an estimate for a limit of guaranteed purity (2). Therefore, the values reported here represented a range within a specified precision for the standards, where the analytical method and the instrumentation were reliable for low-level determinations (6). The levels of solvent residues, ranging from 30 to 300 ppm, obtained during the film-coating process were statistically significant. However, after 24 h of drying, no peaks for either solvent were observed in the chromatograms.

This procedure is easy and reliable for monitoring organic residues on film-coated tablets. Determining lower limits of detection should be an integral part of any statistical package developed for a new analytical method.

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High-Performance Liquid Chromatographic Method for the Determination of Trace Amounts of Acetaminophen in Plasma

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Received July 15, 1982, from the *Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Corporation, Bloomfield, NJ 07003.* Accepted for publication November 5, 1982.

Abstract □ A method is described for the rapid, sensitive, and precise quantitation of acetaminophen in human plasma. The assay involved a single acetonitrile extraction and high-performance liquid chromatographic analysis using a reverse-phase column, with a mobile phase of methanol and water. The limit of quantitation of acetaminophen by this method was 8 ng/mL; only 0.1 mL of the plasma sample was required for the determination. *N*-Propionyl-*p*-aminophenol was used as the internal standard.

Keyphrases □ Acetaminophen—high-performance liquid chromatography determination of trace amounts in plasma □ High-performance liquid chromatography—method for the determination of trace amounts of acetaminophen in plasma

Although acetaminophen is currently one of the most commonly used analgesics and antipyretics, hepatotoxicity has occurred in cases of acetaminophen overdose. Thus, a simple and rapid method for determining acetaminophen concentrations in plasma would be useful.

At present the most sensitive methods for plasma acetaminophen determination are liquid chromatography with electrochemical detection (1) or UV detection (2–6) and GC (7, 8). The quantitation limit for each of these methods is ~100 ng/mL with a 1-mL sample of plasma or serum. These methods, therefore, may be inadequate for microliter samples such as those obtained from newborn infants. Because of time requirements they may not be rapid enough for the determination of acetaminophen in overdose cases. A rapid, specific, high-performance liquid chromatographic (HPLC) method for the determination of acetaminophen in plasma using a 100-μL sample is described in this paper.

EXPERIMENTAL

Reagents and Materials—Acetaminophen[1] standard solutions were prepared in ethanol (1.0 mg/mL, stored at 4°C) and subsequent dilutions

[1] U.S. Pharmacopeial Convention, Inc., Rockville, Md.

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