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Note

Gas chromatographic determination of acetone in hydroxyethyl starches

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Hydroxyethyl starches (HESs), which are used medically as plasma volume expanders and leukapheresis, are made by alkaline ethoxylation of starch followed by neutralization with hydrochloric acid. Ethylene glycol[1] and chloroethanol are possible side products of the process mandating a clean-up step. The present clean-up procedure includes a series of acetone washes of the aqueous slurry. A method capable of determining the trace levels of acetone in HESs down to at least 100 ppm and suitable for routine quality control use was desired.

Numerous methods exist in the literature for the determination of acetone. During the past two decades these methods have consisted primarily of high-performance liquid chromatography (HPLC)[2–5] and gas chromatography (GC)[6–13]. Unfortunately, the HPLC methods require specialized columns[2], fail to state the limit of detection[3,4], or require time-consuming evaporation/concentration steps[4,5], making them unsuitable for routine quality control (QC) use. The GC methods suffer from similar limitations. Several methods are concerned with exploration of column packing for the identification of low-molecular-weight ketones[6–9]. Others require time consuming solvent preparation[10,11] or specialized techniques[12], making them inappropriate for routine QC use. One method was specifically designed for use in routine QC testing, however, it was designed for samples containing substantial amounts of acetone, not trace level determinations, and was not suitable for use with water-soluble polymers as it would require direct injection of the HES onto the column[13].

This paper describes a procedure for determining the acetone content of the water-soluble polymer, HES, suitable for routine QC use. It does not require solvent clean-up or specialized techniques.

EXPERIMENTAL

Materials

HES (American McGaw, Irvine, CA, U.S.A.) was used as received. Acetone and phenylhydrazine (Aldrich, Milwaukee, WI, U.S.A.), methyl ethyl ketone, toluene and *o*-xylene (Burdick and Jackson, Muskegon, MI, U.S.A.), were used as received. Deionized water was further purified (Milli-Q water system; Millipore, Bedford, MA, U.S.A.) prior to use.

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Apparatus

Gas chromatographs equipped with flame ionization detectors and a laboratory data system (Hewlett-Packard, Avondale, PA, U.S.A.) were used. The GC conditions were as follows: a 6 ft. × 4 mm glass column, packed with 3% OV-1 on Chromosorb W HP 100–120 mesh, with temperature set at 150°C for the inlet, 125°C for the column, 300°C for the detector, and using helium as the carrier gas at a flow-rate of 50 ml/min.

An alternative temperature program of the column temperature yielding the same results is as follows: 125°C for 23 min —temperature increased to 175°C at 30°C/min and then held at 175°C for 5 min.

Standards

A stock standard solution was prepared by transferring exactly 2.0 ml of acetone to a 200-ml volumetric flask and diluting with water. A 1- or 2-ml volume of this solution was diluted to 10 ml in water to yield working stock standards. A set of calibration standards was prepared by dilution with water.

A stock internal standard solution was prepared by transferring 2.0 ml of methyl ethyl ketone (MEK) to a 200-ml volumetric flask and diluting with water. A 1-ml volume of this solution was diluted in turn to 10 ml with water to yield a working stock internal standard. Exactly 15.0 ml of the working stock internal standard and 10 g of sodium acetate were transferred to a 100-ml volumetric flask to yield an internal standard solution of 120.75 μg/ml MEK and 10% (w/v) sodium acetate.

Procedure

Into a 15-ml screw-cap culture tube was placed 400 mg of HES and 2.0 ml of water. The sample was mixed by vigorous agitation until complete dissolution was effected. A 1-ml volume of a 0.1 *M* phenylhydrazine hydrochloride solution (1.44 g/100 ml), 1.0 ml of internal standard solution and 4.0 ml of *o*-xylene were added to a culture tube containing 2.0 ml of sample or standard preparation. The tube was sealed with a PTFE-lined screw cap, shaken for 10 min and centrifuged to break the emulsion that formed. A portion of the upper-layer (*o*-xylene) was placed into a autosampler vial for injection into the gas chromatograph.

RESULTS AND DISCUSSION

Methodology

Hydroxyethyl starches are polymers with molecular weights ranging from 50,000 to over 2,000,000. Consequently, it was inadvisable to inject HES solutions directly into a GC system. HES is a water-soluble polymer with no appreciable solubility in organic solvents. In order to ensure that no entrapped acetone was missed, complete dissolution of the HES was desirable. Therefore, it became necessary to derivatize the acetone to permit its extraction from the HES.

Initially, attempts were made to use 2,4-dinitrophenylhydrazine to make the chromatographic derivative, as had been reported in the literature[6,11,12]. Due to the poor quality of the reagent it was found that good derivatives with clean chromatograms were not consistently obtainable and, since a quick, simple QC procedure was needed, clean-up of the reagent was not considered. Phenylhydrazine, which was also

reported in the literature[10], was tested. Problems again were encountered with the derivatizing agent. The phenylhydrazine from Mallinckrodt was found to be unacceptable. The phenylhydrazine from Aldrich was found to yield good derivatives consistently and was used for all work. The phenylhydrazine solution was found to be usable for about one week.

The structural identity and integrity of the derivative was confirmed by GC–mass spectrometry (MS) using chemical ionization.

Since it was our desire to avoid solvent clean-up, potential extraction solvents were evaluated by performing the experiment and varying only the solvent. Lack of interference, recovery, and stability of the derivative were the criteria upon which these solvents were evaluated. Only *o*-xylene was found to be a satisfactory extraction solvent. Benzene, carbon tetrachlroride, chloroform, cyclohexane, ether, ethyl acetate, hexane, and methylene chloride were found to be unsuitable.

The peaks in the chromatogram (Fig. 1), which elute after the peaks of interest

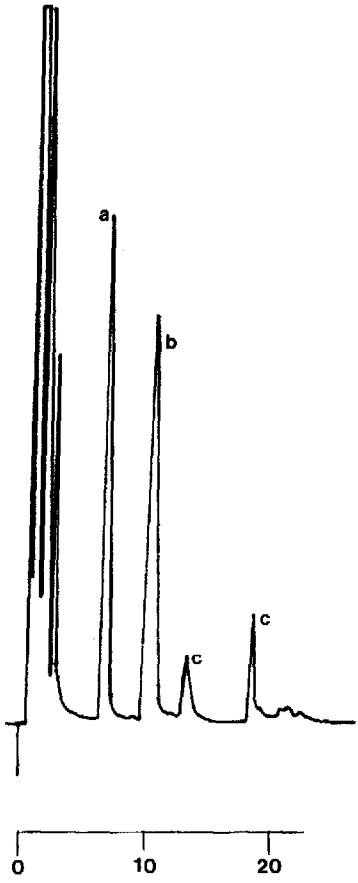

Fig. 1. Typical chromatogram (temperature programmed). Peaks: a = acetone phenylhydrazone; b = methyl ethyl ketone phenylhydrazone; c = unknowns traceable to the reagents used.

TABLE I

ACETONE LEVELS IN FOURTEEN SAMPLES OF HES

A–J are low-molecular-weight HES; K–N are high-molecular-weight HES.

Lot	*Acetone mean value (ppm)*	*Number of determinations*
A	365.8	3
B	405.3	2
C	970.0	2
D	333.7	2
E	893.6	2
F	827.4	2
G	945.1	4
H	646.6	2
I	558.7	4
J	125.2	4
K	114.0	3
L	457.2	3
M	306.3	4
N	402.3	4

TABLE II

METHOD REPRODUCIBILITY (SINGLE ANALYST)

Replicate	*Acetone (ppm)*
1	715.9
2	701.4
3	745.0
4	770.2
5	710.7
6	729.5
7	719.3
8	702.5
Mean	724.3
Relative percent standard deviation	3.2%

TABLE III

DETERMINATIONS OF ACETONE LEVELS IN FIVE LOTS OF HES (IN ppm) BY TWO ANALYSTS

Lot	*Acetone (ppm)*	
	Analyst A	*Analyst B*
K	115.9	114.0
J	175.9	125.2
B	362.9	405.3
C	857.7	970.0
L	482.1	457.2

were identified as resulting from phenylhydrazine and *o*-xylene, respectively, and were present in all lots tested.

Buffers were examined for their effect on the derivatization and extraction. Sodium acetate was chosen because it yielded the largest peak area for the phenylhydrazone of acetone in the chromatogram. An evaluation of various amounts of sodium acetate revealed that 10% (w/v) was optimum. The extraction time was examined and a time of 10 min was found to be best.

At the maximum sensitivity setting consistent with satisfactory instrument responses a detection limit of 4 ppm acetone in HES was achieved. The limit of quantitative determination was found to be 40 ppm. Greater sensitivity is theoretically available by evaporation of the *o*-xylene.

Sets of standard curves were run on eighteen different days. An intercept not significantly different from zero was found. An accuracy of at least 95% and a relative percent standard deviation of less than 6% was seen for all concentrations. Correlations greater than 0.998 were consistently observed.

Analysis of HES samples

The peak area for the phenylhydrazone derivatives of acetone and MEK were measured automatically by chromatographic data systems. The peak area ratios (acetone phenylhydrazone/MEK phenylhydrazone) for the standards were used to construct a least square calibration line. The calibration line and the peak area ratios for the samples were used to determine the concentration of acetone in HES.

Eight lots of low-molecular-weight HES (weight-average molecular weight, $\bar{M}_w$ = 150,000–350,000) and four lots of high-molecular-weight HES ($\bar{M}_w$ = 350,000–500,000) (Table I) were examined to illustrate the applicability of the method. The reproducibility of a single analyst was also explored (Table II). Five lots of HES were examined by two analysts on separate days (Table III). These results show reasonable analyst-to-analyst reproducibility. Better reproducibility was probably not obtainable due to the non-homogeneity of the acetone content of HES, which may vary depending upon the location of the sampling from the storage container.

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