zinc stearate had an average particle size of less than 9 μ and that calcium stearate average particle size was substantially less than this. Magnesium stearate ranged from 10–50 μ by microscopic examination and showed many long, feathery type of particles. Whether these differences in particle size are of significance in the lubrication efficiency has not yet been determined. The other factor in question with regard to the wet process is the degree of dispersibility of the stearates in isopropanol.

CONCLUSIONS

The measurement of lower-punch pulldown force (LPPF) has been demonstrated on the two rotary tablet presses. The LPPF measurement is a qualitative or semiquantitative means of detecting changes in frictional forces involving lower-punch movement. It has utility in assessing frictional aspects of tooling, formula lubrication, and aids in prevention of machine breakdown or excessive tool wear. Similar research on upper cam-track measurement is now in progress.

REFERENCES

(1) E. L. Knoechel, C. C. Sperry, H. E. Ross, and C. J. Lintner, *J. Pharm. Sci.*, **56**, 109(1967).

(2) E. L. Knoechel, C. C. Sperry, and C. J. Lintner, *ibid.*, **56**, 116(1967).

(3) P. E. Wray, J. G. Vincent, F. W. Moller, and G. J. Jackson, paper presented to the Industrial Pharmacy Section, APhA Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

(4) W. O. Strickland, Jr., *Drug Cosmetic Ind.*, **85**, 319(1959).

(5) J. J. Windheuser, J. Misra, S. P. Eriksen, and T. Higuchi, *J. Pharm. Sci.*, **52**, 767(1963).

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NOTES

Determination of Hexachlorophene in Whole Blood

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Abstract □ An efficient method for the quantitative recovery of hexachlorophene from whole blood is described. It can be applied to both human and animal blood, and utilizes an extraction procedure followed by electron-capture detection with gas chromatography. Problems encountered in the reproducible isolation of hexachlorophene are discussed. Recoveries are in the 90–100% region with detectable levels down to 0.03 p.p.m. No blank interferences are observed.

Keyphrases □ Hexachlorophene—analysis in blood □ Extraction procedure—hexachlorophene in blood □ GLC electron-capture—analysis

A method for the quantitative analysis of hexachlorophene in animal tissues including blood has previously been described (1). The extraction and cleanup techniques were time consuming, and the lower limit of sensitivity was approximately 20 mcg. A recent publication demonstrated that hexachlorophene could accurately be determined at subnanogram levels using electron-capture gas chromatography (2). An efficient procedure for extracting hexachlorophene from blood and an improved analysis of the extract by electron-capture gas chromatography is described in this report.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a helium-discharge electron-capture detector was used in this work. A mixer[2] using 40-ml. round-bottom centrifuge tubes proved suitable for the extractions.

Reagents—Anhydrous ethyl ether, reagent grade hexane, and Tri-Sil concentrate[3] are used.

Procedure—*Calibration Curve*—One gram of hexachlorophene was dissolved in 100 ml. of ethanol. Ten microliters of this solution was diluted to 10 ml., and this dilution was used to prepare the calibration curve. Aliquots of 20, 40, 60, 80, and 100 μl. were evaporated to dryness and 10 μl. Tri-Sil concentrate was then added to the residue, followed by 1 ml. of hexane. One-microliter volumes were injected into the chromatograph. A calibration curve was prepared by plotting concentration *versus* recorder response (I_B background current) (2). The assay was thus calibrated from 0.2–1.0 ng./μl.

Recovery from Blood—Known amounts of hexachlorophene in alcoholic solution, as in the calibration procedure, were added to 3 ml. of whole blood contained in 40-ml. round-bottom centrifuge tubes. Seven milliliters of distilled water was then added to thin the blood and facilitate the thorough incorporation of hexachlorophene into the blood. This was followed by five successive 10-ml. extractions with ethyl ether, using the mixer. The blood-ether layers

[1] Beckman GC-5, Beckman Instruments, Inc., Fullerton, Calif.
[2] Vortex Genie, Scientific Industries, Inc., Springfield, Mass.
[3] Pierce Chemical Co., Rockford, Ill.

Table I—Typical Recoveries

Sample	Hexachlorophene Added, ng.	Found, ng.
Human blood	120.0	113.0
Human blood	60.0	57.0
Human blood	5.0	5.0
Human blood	3.0	2.8
Human blood	3.0	2.8
Human blood	0.60	0.58
Sheep blood	4.0	4.0
Sheep blood	3.0	3.0
Dog blood	10.0	9.5
Dog blood	5.0	4.8

were centrifuged, and the top layers were drawn off with a capillary pipet into another 40-ml. centrifuge tube. Then the combined extracts were evaporated to dryness on a hot-water bath. The residue obtained was made alkaline with 5 ml. of 5% aqueous NaOH, and this solution was successively extracted with four 10-ml. portions of hexane, again using the mixer. The hexane-alkaline layers were centrifuged so that hexane could be drawn off and discarded. Residual hexane was evaporated by warming on a hot-water bath. The aqueous layer obtained was then acidified with 5% aqueous HCl and extracted twice with ether. After centrifugation the ether layers were drawn off into a 40-ml. round-bottom tube and evaporated to dryness. All traces of ether must be completely removed. (Oxygenated solvents seriously reduced the sensitivity of electron-capture detectors.) Ten microliters of Tri-Sil concentrate was added to the tube, followed by 1 ml. of hexane. One microliter of this solution was injected into the chromatograph.

RESULTS AND DISCUSSION

Table I contains a listing of typical recoveries. Recoveries for both human and animal blood are usually in the 90–100% range. Precision is in the order of 1.3% relative standard deviation as calculated from the values in Table II. Recoveries are comparable whether extractions are made immediately after adding hexachlorophene or after storing the blood in a freezer.

The first ether extraction of a citrated blood may form an indistinct interface due to emulsion with the blood, but subsequent ether extractions will improve the interface so that the ether recovery is quantitative with minor losses.

Table II—Replicate Analyses of Hexachlorophene

Added, ng.	Recovered, ng.
600	560
600	560
600	570
600	550
600	560
	Mean 560

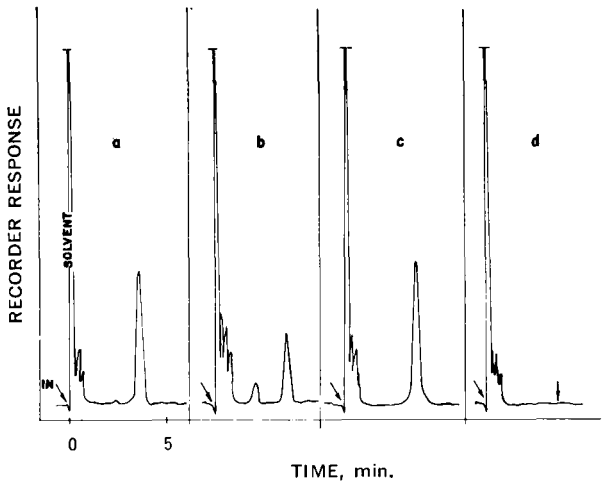

Figure 1—a, *0.5 ng./μl. of hexachlorophene-trimethylsilyl ether standard;* b, *0.5 ng./μl. response in the presence of ether solubles from blood;* c, *cleanup response showing noninterfering volatiles present in blood;* d, *control blood showing no response in the hexachlorophene area.*

It is essential that the final extract be free from oils or other residues so that quantitative silylation can occur. Figure 1*a* shows the response of 0.5 ng./μl. standard hexachlorophene-trimethylsilyl ether. Figure 1*b* shows the same quantity after a 1-ml. hexane solution of interfering extractables was intentionally added and the procedure carried out to completion. A loss of approximately 64% is observed. The cleanup procedure in which the sodium salt of hexachlorophene is formed eliminates most interferences. Figure 1*c* shows the response after cleanup. Here there is evidence of other volatiles which elute before hexachlorophene-trimethylsilyl ether but do not interfere in recovery. A blood control having no hexachlorophene is shown in Fig. 1*d*. No interferences are observed.

The method as described, with perhaps minor variations, should be directly applicable to other body fluids such as urine or serum. Indeed, suitable isolation from biological tissue may involve such an extraction procedure as well. This will be the subject of further investigations.

REFERENCES

(1) V. D. Johnston and P. J. Porcaro, *Anal. Chem.*, **36,** 124 (1964).

(2) P. J. Porcaro and P. Shubiak, *ibid.*, **40,** 1232(1968).

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