NEW APPARATUS

A CONTINUOUS EXTRACTOR FOR USE IN TOXICOLOGICAL ANALYSIS

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A continuous extractor using ethanol under reduced pressure has been developed for the isolation of many poisons from viscera. Barbiturates, alkaloids and glycosides have been investigated.

IN the many processes suggested over the last hundred years for the extraction of poisons that are soluble in organic solvents, ethanol has figured as the solvent most used in the initial extraction scheme. The original method Stas and Otto has been modified by many workers and is still used exclusively in many countries. Notwithstanding the fact that the process is lengthy and somewhat tedious it must be used in parts of the world where, because of the high temperatures, ethanol is used as a

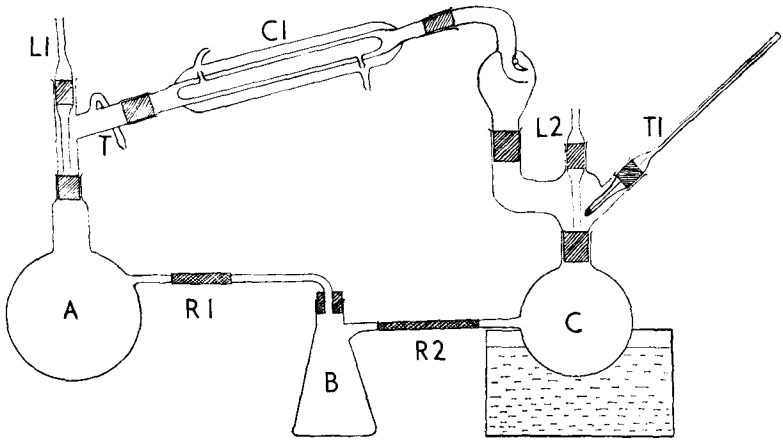

FIG. 1.

preservative. The alternative methods for the extraction are therefore not available to these toxicologists. Although Umberger[1] has described a method using continuous extraction of tissue with ethanol, unfortunately his device is complicated and not readily available commercially. We have therefore developed the apparatus shown in Figure 1. It is a continuous extractor which works under reduced pressure and uses ethanol as the extracting solvent. The tissue, liver, brain or contents of the alimentary tract, is macerated in a blender with 95 per cent ethanol and placed in the large flask "A" of either 1 or 2 litre capacity. The 1 litre extraction flask conveniently handles 100 to 150 g. samples of tissue. The 2 litre flask is adequate for 500 g. samples.* The ethanol level is then

* The apparatus is available from Messrs. Woods Bros., Glass Company Limited of Barnsley (Yorkshire) in two sizes (1 and 2 litre extraction flasks).

adjusted so that it just overflows into a setting tank (the conical flask "B"). This conical flask is filled with absolute ethanol and as the supernatant from the extraction flask overflows into it further precipitation of protein occurs. The precipitate settles readily to the bottom of this flask and does not reach the evaporation flask (flask "C") which is of 500 ml. or 1 litre capacity depending on the size of flask "A". When the 1 litre size evaporating flask is used it must be lengthened with a 3 in. adaptor to compensate for the increased size of the 2 litre extraction flask. The overflow from the settling tank refills the evaporation flask which has been previously filled to about one inch above the sidearm with 95 per cent ethanol. The rubber connectors (R1 and R2) are joined, the air leaks (L1 and L2) drawn out in a flame to fine capillaries. Suction is then applied (via T) from a water pump.

The exact pressure depends on the ambient room temperature. In England this is about 20° and the required pressure is then about 10 cm. of Hg. It is important to connect a simple manometer to the pump so that an exact pressure reading can be taken. At 10 cm. of Hg, ethanol boils at approximately 30° and, with the lower part of the evaporation flask surrounded by a water bath at 80°, the rate of boiling in the flask "C" is then rapid and controlled. The ethanol vapour condenses (condenser C1) and is returned to the extraction flask. Cycling of the ethanol is thus achieved and continuous extraction of the tissue and concentration of the extract in flask "C" established.

The pressure must not be reduced to such a degree that there is a gross loss of ethanol down the water pump. We would suggest that the pressure should be adjusted in each case by means of a capillary leak on the pump so that about a 12° temperature differential is maintained between room temperature and the evaporation temperature of the ethanol (thermometer T1).

Periodic agitation of the ethanol in flask A is accomplished by means of a screw clip on a rubber tube attached to the air leak (L1).

Extraction Times

We first demonstrated the efficiency of the apparatus by adding phenolphthalein to 100 g. of liver in the 1 litre extraction flask. This provided a simple colorimetric method of following the extraction by observing the red colour produced by adding aqueous sodium hydroxide to aliquots of ethanol taken from each of the flasks A, B and C. We found that 2 hours were necessary for complete transfer of the phenolphthalein from flask "A" to flask "C". Henceforth we used a 6 hour period for the extractions. No ethanol had to be added during this period, the apparatus worked perfectly without attention.

Compounds Investigated

Barbiturates

100 g. of liver from a case of amylobarbitone poisoning and 2 g. of tartaric acid were macerated with ethanol and extracted in the apparatus.

The contents of flasks "B" and "C" were then filtered through a Whatman No. 4 fluted filter paper and the filtrate evaporated under water pump pressure (15 mm.) to approximately 10 ml. 100 ml. of 0·5 N sulphuric acid was then added and the solution refiltered. The barbiturate was then extracted from the aqueous acidic solution with ether (200 ml.) which was washed with 20 ml. of 5 per cent aqueous sodium bicarbonate solution and then shaken twice with 20 ml. of 2·5 per cent aqueous sodium hydroxide. The alkaline aqueous extracts were acidified and re-extracted with ether. The ether extract was evaporated and the barbiturate assayed by ultra-violet spectrophotometry[2]. Comparison of the recovery using the continuous extractor with the recovery of barbiturate from the same liver using a tungstic acid method[3] showed that the extractor gave an approximately 90 per cent recovery compared with the latter method.

Alkaloids

The extraction of four alkaloids was investigated. Quinine was chosen because its high ultra-violet absorption spectrum at 347 mμ made it especially suitable for accurate spectrophotometry. Morphine was next

TABLE I

RECOVERY OF ALKALOIDS

Viscera (100 g.)	Alkaloid	Alkaloid added, mg.	Recovered, mg.	Recovery, per cent
Liver	Quinine	3·0	2·0	66
Liver	Morphine	1·5	1·2	80
Liver	Atropine	3·0	2·7	90
Brain	Cocaine	3·0	1·54	51

investigated because it is very commonly encountered and because it is an alkaloid which often presents special difficulties in extraction. Finally atropine and cocaine were added to viscera and their extraction followed. These last two compounds contain ester linkages which make them very easily hydrolysed.

The procedure for isolation was identical with that of the barbiturates described above up to and including the extraction by ether of the aqueous sulphuric acid. The aqueous phase was then made alkaline with ammonia and shaken in turn with ether and chloroform : isopropanol (5 : 1). Quinine and cocaine were estimated by ultra-violet spectrophotometry after evaporating the ether phase and dissolving aliquots of the extract in 0·1 N sulphuric acid (quinine 347 mμ) (cocaine 233 mμ) and atropine and morphine by comparative paper chromatography on aliquots of the ether and chloroform isopropanol phases respectively. The solvent system in each case was that of Curry and Powell[4]: the method of detection was Dragendorff's reagent for atropine and Marquis reagent for morphine. Paper chromatography was also used to cross check the ultra-violet spectrophotometric results for quinine and cocaine. The viscera used were liver and brain and the results are tabulated in Table I.

Glycosides

We considered that this method of extraction might be suitable for the isolation of glycosides. This is a subject which has been largely ignored in the last 20 years in toxicological literature. Digoxin and solanine were added to the contents of a stomach and small intestines and Table II shows that the results are highly encouraging.

The isolation identification and assay of these glycosides was achieved by the following procedure.

As with the barbiturates and the alkaloids, the ethanol in the extraction flask was evaporated under water pump pressure to about 10 ml. Water (100 ml.) and dilute sulphuric acid (10 ml.) were added and the turbid solution was filtered. After adjusting to pH 5 digoxin was extracted from the aqueous solution by two extractions with 100 ml. of chloroform which was separated off and evaporated. Solanine was then extracted with warm pentanol after making the aqueous phase slightly alkaline with ammonia. This alcohol was also evaporated under reduced pressure.

TABLE II

RECOVERY OF GLYCOSIDES

Viscera	Glycoside	Added, mg.	Recovered, mg.	Recovery, per cent
Stomach and small intestine contents	Digoxin	7·5	5·5	73
Liver (100 g.)	Digoxin	2·5	2·0	80
Stomach and small intestine contents	Solanine	10·0	4·0	40

Proof of identity and quantitative assay were achieved as follows. The extract containing digoxin was dissolved in 70 per cent aqueous ethanol and aliquots were examined by paper chromatography in the following solvent systems[5,6]:

(*a*) chloroform : methanol : water (10 : 2 : 5) (organic layer used);

(*b*) chloroform : benzene : water (65 : 35 : 50) + 7·5 ml. methanol (organic layer used).

The first spray was 5 per cent *m*-dinitrobenzene in benzene. After heating in the oven at 80° for 15 minutes the paper was dipped in 20 per cent sodium hydroxide solution. Digoxin showed as a blue spot at R_F 0·71 in solvent (*a*) and R_F 0·32 in solvent (*b*). Two other methods of detection were sprays of 5 per cent trichloracetic acid and 20 per cent antimony trichloride in dry, alcohol free, chloroform; in each case the paper was heated at 80° for 3 minutes after spraying. We felt that further proof of identity might be desirable and so investigated the chromatography of digoxigenin. We found this aglycone gave a green fluorescence under ultra-violet light on the solvent front (solvent system (*a*)) when the paper was sprayed with the antimony trichloride reagent and heated at 70° for 3 to 5 minutes. We demonstrated the conversion of the recovered digoxin to digoxigenin by warming with dilute hydrochloric acid at 100° for 30 minutes. We also showed that the apparatus and method outlined above gave excellent recovery of digoxin added to liver but that when digoxigenin

was added to liver it could not be recovered. We have as yet no explanation for this finding.

Solanine was identified and estimated by a comparative chromatogram using Curry and Powell's system[4] and Clarke's reagent[7] (1 per cent paraformaldehyde in concentrated phosphoric acid) poured on to the paper. The solanine was recovered unchanged; there had been no loss of the sugars to give solanidine.

Discussion

An apparatus has been developed for the extraction of many classes of poisons from viscera. Ethanol was chosen not only because it is a good solvent for the poisons, but because in many countries the viscera are submitted to the laboratory already preserved in it.

The efficiency of the extraction has been followed using the barbiturates, alkaloids and glycosides. Substances were chosen which were either insoluble in the usual solvent systems and did not fit in the general extraction scheme or were substances whose instability was such that their extraction was particularly difficult.

In all forensic work speed is often of vital importance and we suggest that this extractor will materially reduce not only the time required but also the volume of ethanol necessary for the extraction of many poisons.

In the examples discussed above, we deliberately added milligram quantities of poison to the viscera: however, the detection and assay were always accomplished with less than 1 per cent of the extract and the scheme should be applicable in cases of poisoning by these classes of poison.

It seems probable that such an apparatus might be of use in many other branches of chemistry.

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