Z Rechtsmed (1983) 90 : 81–85

Zeitschrift für
Rechtsmedizin

Originalarbeiten / Original Works

Application of a Radiometric Method for Evaluation of Loss of Salicylic Acid During Isolation from Biologic Material

A. Ostrowski

Institute of Forensic Research, ul. Westerplatte 9, PL-31-033 Kraków, Poland

Summary. A radiometric method for evaluation of loss of salicylic acid in the process of isolation from biologic material is described. According to this study the mean loss during the total process of isolation amounts to 33.59%, the specific values being 19.47% during protein precipitation, 10.68% during extraction, and 3.44% during evaporation of solvent.

Key word: Radiometric evaluation, isolation of salicylic acid in biologic material

Zusammenfassung. Es wird eine radiometrische Methode zur Schätzung der Salicylsäureverluste bei der Isolierung aus biologischem Material angewandt. Die Untersuchung ergibt, daß der mittlere Verlustwert bei 33,59% liegt, wovon 19,47% auf Enteiweißung, 10,68% auf Extraktion und 3,44% auf die Abdampfung des Lösungsmittels entfallen.

Schlüsselwort: Radiometrische Methode, Isolierung von Salicylsäure in biologischem Material

Introduction

Isolation of poisons from biologic material is still a question of topical interest to be answered (Bremer 1976; Grusz-Harday 1965). Particularly important from the analytic view point is the problem of loss of separated substances (Borkowski and Ostrowski 1973, 1974; Curry and Phang 1960; Tompsett 1968).

This work aims at introducing a radiometric method for the evaluation of separation efficiency of organic compounds from biologic material. Special attention has been paid to the possibilities of loss, their extents and causes in different stages of the isolation process.

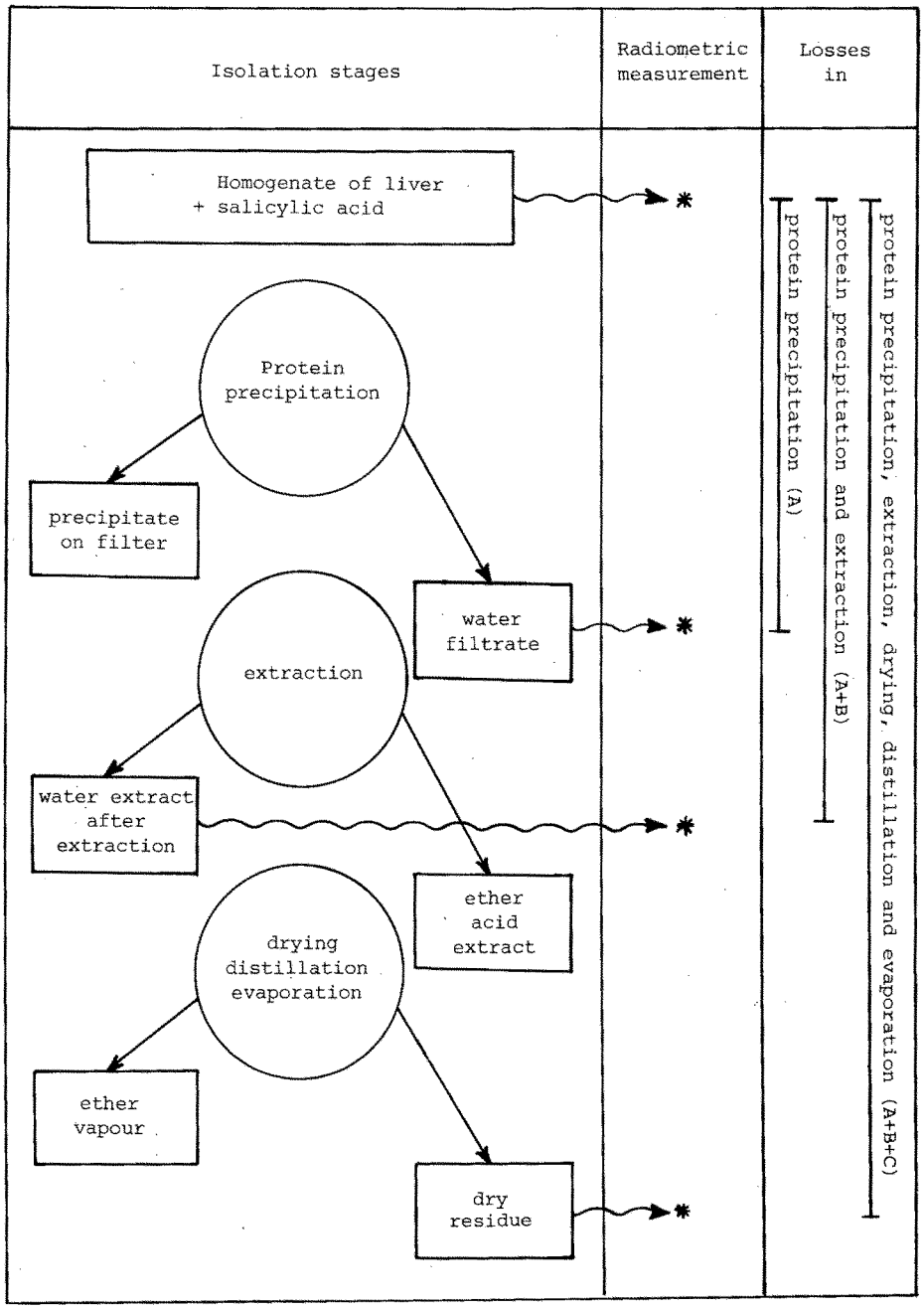
Isolation stages
Radiometric measurement
Losses in
Homogenate of liver + salicylic acid
Protein precipitation
precipitate on filter
water filtrate
extraction
water extract after extraction
ether acid extract
drying distillation evaporation
ether vapour
dry residue
protein precipitation (A)
protein precipitation and extraction (A+B)
protein precipitation, extraction, drying, distillation and evaporation (A+B+C)

Fig. 1

Materials and Methods

Beta-radioactive salicylic acid marked with the ^{14}C carbon isotope with the specific activity of 61 mCi/mmol was used as a standard compound. The experimental material consisted of the livers from corpses of persons who had not taken any drugs, in particular salicylic acid, prior to their death.

Radioactive salicylic acid was added to 50-g samples of homogenized human liver, then the samples were maintained at 4° C for 24 h to assume the complete balance of concentrations and the protein-salicylic acid equilibrium. The separation of salicylic acid from the homogenates was carried out using the ammonium sulfate method (Borkowski 1968). Thirty grams ammonium sulfate was added to each liver homogenate, and the samples were acidified to pH = 4 with 2 *N* sulfuric acid. The acidified homogenates were boiled for 3 min and the separated precipitate was filtered through a paper filter moistened with water. The precipitate on the filter was thoroughly washed three times with boiling water, yielding 700 ml of the filtrate. The obtained clear filtrate represented the protein-free aqueous liver extract.

This extract, after the process of protein precipitation, was acidified to pH = 1 with 5 *N* sulfuric acid, transferred into a modified Kutscher-Steudel apparatus (Weisberger 1956) and continuously extracted with 400 ml of ether for 12 h, yielding an acidic ether extract, which finished the extraction stage.

The extract was dried with 40 g of anhydrous sodium sulfate, which was filtered off after 1 h and washed on the filter with ether, yielding 400 ml volume of the filtrate. The dry extract was concentrated by distillation to a 100-ml volume and transferred to an evaporation dish together with a few portions of ether used for washing the distillation flask. When the spontaneous evaporation of the solvent was completed the dry residue was transferred into a 25-ml volumetric flask, using acetone as a solvent. Figure 1 shows the isolation process.

The determinations were performed using a scintillation counter (Intertechnique–Paris, Model SL-30) equipped with an external standard (137-Cs). The following liquid scintillator

Table 1. Comparison of the losses of salicylic acid in separate isolation stages

	Isolation stages		
	Protein precipitation (%)	Extraction (%)	Drying, distillation, evaporation (%)
	A	B	C[a]
Number of sample			
I	18.42	8.89	4.05
II	18.67	14.21	−1.23
III	17.78	9.22	7.32
IV	21.18	11.53	−1.07
V	18.15	8.01	9.06
VI	22.64	12.19	2.50
$\bar{X}$	19.47	10.68	3.44
SD	2.00	2.34	4.24
CV	10.27	21.94	123.26

[a] The values in column C are the differences between the total amount of losses (A + B + C) and the sum of losses given in columns A and B. C = (A + B + C) − (A + B)

$\bar{X}$ = mean value; SD = standard deviation; CV = coefficient of variation in percent

was used: 5 g PPO (2,5-diphenyloxazole) and 100 mg dimethyl-POPOP (1,4-di-/-2-(4-methyl-5-phenyloxazolyl)-benzene) dissolved in 1000 ml toluene.

Results

The results show that the losses during the whole process of isolation amounted to $\bar{X}=33.59\%$, SD = 2.38%, CV = 7.09%, while for each particular stage of isolation the losses were: $\bar{X}=19.47\%$, SD = 2.00%, CV = 10.27% for protein precipitation, $\bar{X}=10.68\%$, SD = 2.34%, CV = 21.91% for extraction, and $\bar{X}=3.44\%$, SD = 4.24%, CV = 123.26% for drying, distillation, and evaporation (Table 1).

Discussion

As can be seen the most significant loss occurs during the process of protein precipitation from the biologic material. This high percentage of loss may be explained by the possibility of occlusion of salicylic acid by the precipitate of denaturated protein, the formation of protein complexes of biologic material with salicylic acid, and also by technical difficulties associated with the quantitative elution of salicylic acid from the precipitated protein. It is noteworthy that, although the loss during the protein precipitation stage was the highest in comparison with those during the subsequent stages of analysis, its repeatability and consequently the precision of determination were satisfactory.

The results obtained on the evaluation of loss at this stage of the isolation process suggest the need for a modified protein precipitation method toward the reduction of the lost substance with a concomitant preservation or improvement of the precision of determination.

Much lower loss was found during the extraction of salicylic acid from the deproteinized material. Contrary to the repeatable results obtained after the protein precipitation process the determinations of loss during the extraction process were markedly less reproducible and their statistical scatter was much higher. This fact also suggests the need for an improvement of the extraction technique toward a better reproducibility.

The lowest loss was found during the stage of drying, solvent distillation, and evaporation. Although its scatter was rather high, it might be assumed that in the total loss of salicylic acid during the isolation process this finding associated with the final stage has no essential significance.

The results and conclusions presented above prove that the radiometric method can be applied successfully in testing the chemotoxicologic methods used in forensic analysis. They can thus be regarded as a starting point for the continuation of investigations, using radiometry as a research tool, of different steps of chemotoxicologic analysis taking into account metabolic and/or physicochemical processes of the investigated toxic substances.

References

Borkowski T (1968) The method of isolating organic poisons from biological material. Arch Med Sad Krym XVIII: 95–100

Borkowski T, Ostrowski A (1973) A study on the isolation of organic poisons from biological material—Part I. Arch Med Sad Krym XXIII : 149–153
Borkowski T, Ostrowski A (1974) A study on the isolation of organic poisons from biological material—Part II. Arch Med Sad Krym XXIV : 59–63
Bremer H (1976) Isolierungsverfahren einschließlich Aufbereitung des Untersuchungsmaterials. Kriminal Forens Wiss 24 : 174–180
Curry AS, Phang SE (1960) A continuous extractor for use in toxicological analysis. J Pharm Pharmacol 12 : 437–441
Grusz-Harday E (1965) Neues Verfahren zur Extraktion organischer Arzneimittel und Gifte aus Leichenteilen bei toxikologischen Analysen. Mikrochim Acta 1 : 75–80
Tompsett SL (1968) Extraction procedure in chemical toxicology. Analyst (London) 93 : 740–748
Weissberger A (1956) Technique of organic chemistry, vol III, part 1. Interscience, New York

Received in final form August 23, 1982