

AUTOMATIC SAMPLE COMBUSTION METHODS
FOR THE DETERMINATION OF SOFT BETA-EMITTING ISOTOPES
IN DUAL LABELLED ORGANIC COMPOUNDS
AND BIOLOGICAL MATERIALS
BY LIQUID SCINTILLATION COUNTING

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Automatic sample preparation methods for the determination of carbon-14 and/or tritium, and carbon-14 and/or sulfur-35 in dual labelled samples by liquid scintillation counting are presented. The sample is burnt in a stream of oxygen, and the combustion products carrying radioisotopes are subsequently separated and collected for radioactivity determination. Tritium is measured as water, carbon-14 as "carbamate" and sulfur-35 as sulfuric acid. The procedures run automatically, they are free of memory effect and cross contamination, and provide quantitative recovery.

I. INTRODUCTION

The potentialities of tracer experiments using organic substances labelled with soft beta emitting isotopes have been improved by simultaneous application of these radionuclides. However, in chemical and biological experiments involving tritium, carbon-14 and sulfur-35 (e.g. in drug metabolic studies) very often a great variety and a large number of samples must be assayed. Different types of samples may require different sample preparation procedures and utilize different counting solutions. On the other hand, large series of samples can be effectively processed only by using automatic analytical methods. The application of semi or fully automatic sample combustion techniques associated with liquid scintillation counting (Kaartinen, 1969; Peterson et al., 1969; Peterson, 1969; Naokes, 1974; Benakis, 1973; Gács and Dombi, 1978; Gács et al., 1978a, 1978b; Gács et al., 1979), besides decreasing tedious manual labour, provide further advantages as e.g. uniform and favourable final sample composition.

In spite of the developments achieved in the preparation of samples labelled with tritium and carbon-14, however, no rapid combustion methods and suitable instruments have been available for the preparation of multiple labelled organic substances containing other combinations of soft beta emitting isotopes. On the other hand, the improvement of precision and automation as well as reduction of operational and maintenance costs seems also desirable.

With this in view, automatic isotope analytical processes have been developed. Of these methods, the sample preparation techniques used for simultaneous determination of carbon-14/tritium and carbon-14/sulfur-35 in dual labelled organic compounds and biological materials by liquid scintillation counting are presented. For the determination of carbon-14/sulfur-35 an improved version of a previously published method (Gács et al., 1978a) is described. The principles of the procedures are shown in Fig.1 and Fig.2.

In case of materials labelled with carbon-14 and/or tritium (Fig.1), after the addition of a small amount of tungstic oxide to retain phosphorus and promote combustion, the sample is burnt in a stream of oxygen. Halogens and sulfur are retained by silver wool, while the combustion products including HTO and carbon dioxide are led with the oxygen stream onto a column of partially dehydrated alumina kept at 200°C. The water is retained on the column quantitatively, meanwhile the carbon dioxide is transferred into an absorbent suitable for counting. The tritium (HTO) stored temporarily

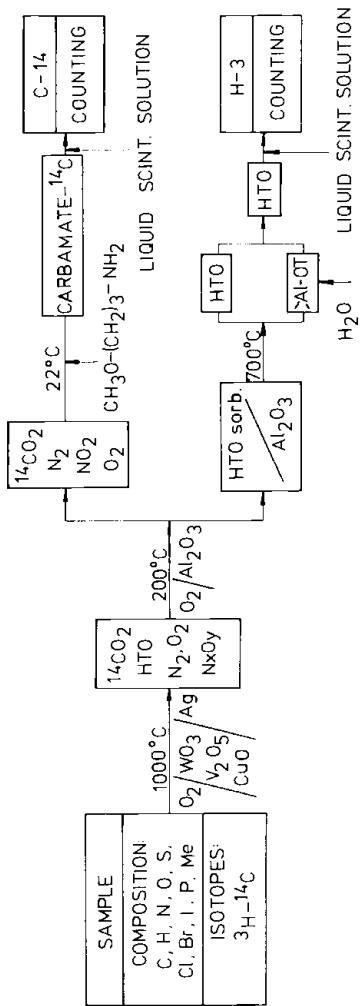


FIGURE 1. Operational diagram for the preparation of samples labelled with carbon-14 and/or tritium.

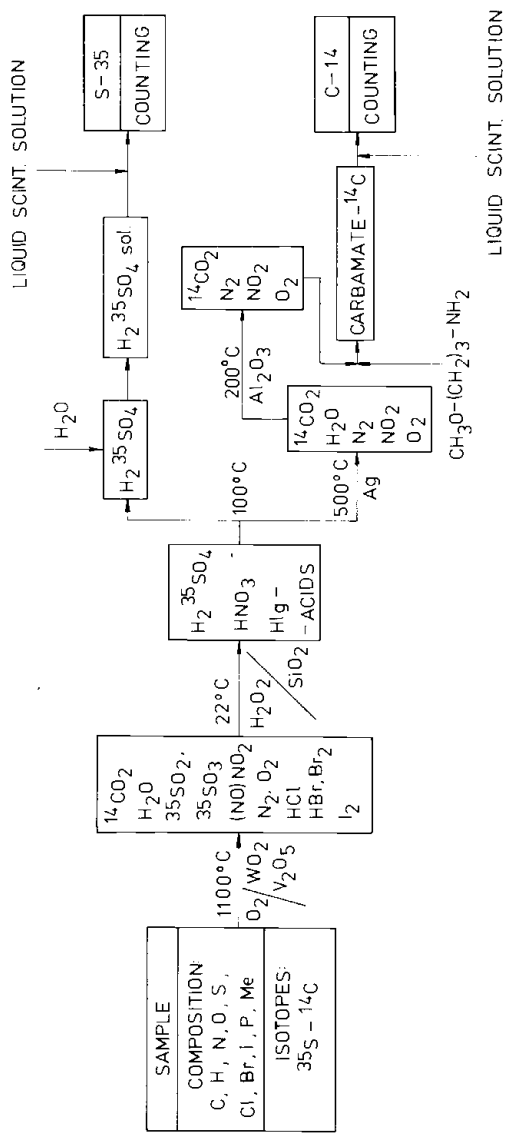


FIGURE 2. Operational diagram for the preparation of samples labelled with carbon-14 and/or sulfur-35

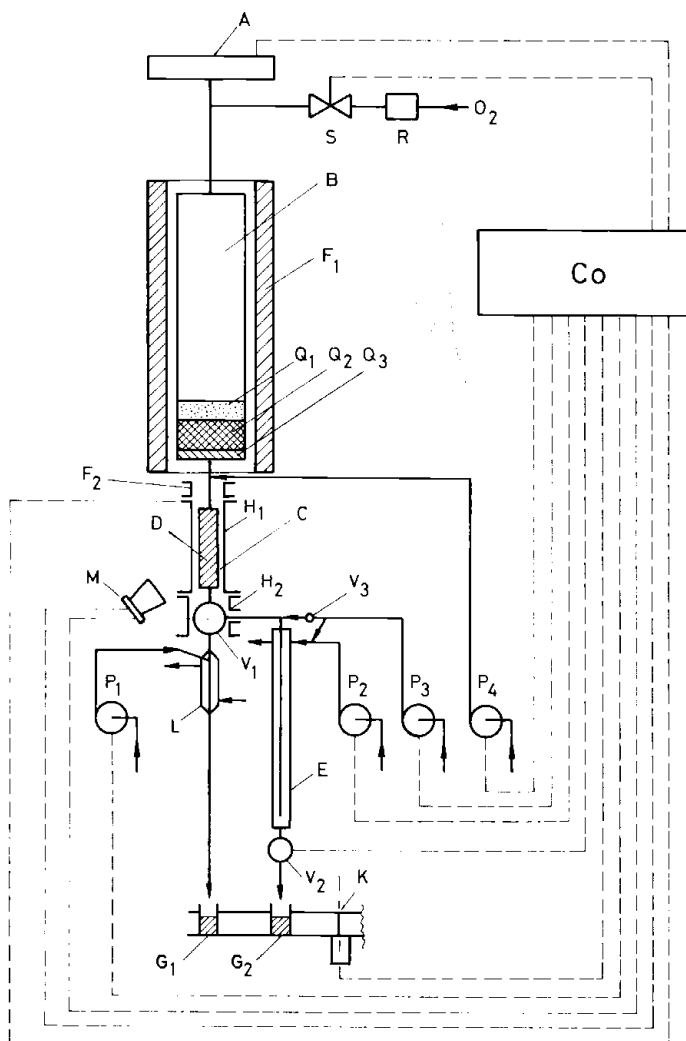


FIGURE 3. Flow diagram of the instrument used for the preparation of samples labelled with carbon-14 and/or tritium

A: sample feeder, B: combustion chamber, C: quartz tube, D: alumina filling, E: absorber, F_1 and F_2 : furnaces, G_1 and G_2 : vials, H_1 and H_2 : heaters, K: vial holder, L: condenser, M: ventilator, P_1 , P_2 , P_3 and P_4 : pumps, Q_1 : quartz chips, Q_2 : copper oxide wire, Q_3 : silver wool, R: flow rate regulator, S: solenoid valve, V_1 and V_2 : stop-cocks, V_3 : flap valve, Co: programmer.

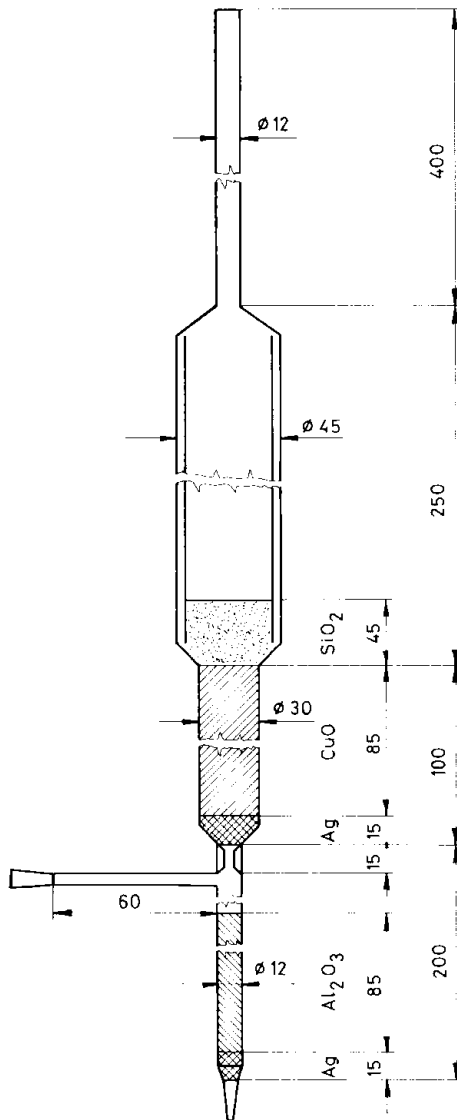


FIGURE 4. Combustion tube.

on alumina is removed without the use of oxygen carrier gas, partly by thermal desorption upon heating the column to 700°C, and partly by rinsing the alumina at the same temperature with a small amount of inactive water fed directly into the system. Preceding combustion of the next sample the alumina is cooled to 200°C. At 200°C the column has abundant capacity to retain water formed from a sample of 250 mg.

In case of materials labelled with carbon-14 and/or sulfur-35 (Fig.2) the sample is also burnt in a stream of oxygen in the presence of tungstic oxide. The sulfur oxides are, however, retained as sulfuric acid on a quartz wool column wetted with dilute hydrogen peroxide (Gács et al., 1977). Other combustion products, including carbon dioxide, pass through the column directly, or on heating the column to 100°C, evaporate into the oxygen stream. Halogens are removed from the gas stream with silver wool, and the carbon dioxide is trapped by an absorbent suitable for counting. The residual sulfuric acid is rinsed off the column with distilled water and the dilute solution obtained is mixed with a liquid scintillation cocktail for radioactivity determination.

II. METHODS

A. Sample Combustion System for Carbon-14 and/or Tritium Determination

1. *Apparatus.* The setup of the apparatus is shown in Fig.3. Sample transferring and feeding device *A* (Gács and Dombi, 1978) which can store 30 samples, drops the samples one by one into combustion chamber *B* heated to 950°C with furnace F_1 . The flow rate of oxygen is adjusted to 150 ml/min with flow rate and pressure regulator *R*. Quartz chips filling Q_1 provides a large contact surface during combustion, copper oxide filling Q_2 (Merck, wire form reagent) ensures complete oxidation of the combustion products, and silver wool packing Q_3 kept at 500°C in the lower part of furnace F_1 removes halogens and sulfur not retained by the copper oxide filling. The water (H₂O) formed from the sample is retained in quartz tube *C* on a column (*D*) of alumina (Applied Science Laboratories Inc., length: 85 mm; d: 10 mm; particle size: 60/80 mesh) maintained at 200°C with heater H_1 . The combustion tube comprising the combustion chamber and the column can be seen in Fig.4. The quartz lining tube is placed in chamber *B* to protect it from possible damage caused during ignition by metal foil capsules holding the samples. The carbon dioxide formed is led into absorber *E* (Gács et al., 1978b) containing

3-methoxypropylamine fed by pump P_2 (see Fig.3). The radioactive solution is rinsed out of the absorber into vial G_2 via stopcock V_2 with a toluene based cocktail (5 g PPO and 0.5 g POPOP in 1000 ml toluene) introduced by pump P_3 . The inner tube of the absorber is washed with the liquid scintillation solution via flap valve V_3 and stopcock V_4 , respectively. The water (H₂O) removed from the alumina partly by thermal desorption at 700°C, and partly by inactive water fed with pump P_4 and subsequently evaporated by means of furnace F_2 , is condensed in condenser L (Fig.5) via stopcock V_1 heated to 100°C with heater H_2 . The water collected in the condenser and an attached tube is rinsed into vial G_1 with a suitable liquid scintillation cocktail (e.g. Aerosol MA/toluene system, Szarvas et al., 1971) pumped by pump P_1 , while alumina column D is cooled to 200°C by means of a stream of air fed with ventilator M . The liquid scintillation vials (G_1 and G_2) are changed automatically with vial transferring device K (Gács et al., 1978b). Sample feeding device A , solenoid valve S , heater H_1 , ventilator M , stopcocks V_1 and V_2 , vial transferring device K and pumps P_1 , P_2 , P_3 and P_4 are actuated and controlled, respectively, by programmer Co (Laborinspector, Chinoin, Budapest). The programmer controls the duration and sequence of the various operations and operational steps, respectively. The duration of each step can be adjusted continuously from one second to ten minutes, or set to infinite. The programmer has ten channels, which are used according to a preset matrix. The dashed lines represent the electrical connections between the programmer and the parts to be actuated. Stopcocks V_1 and V_2 are glass stopcocks operated by small electromotors (Gács and Dombi, 1978).

2. *Procedure.* The sample (up to 85 mg for a single combustion) are weighed into tin or aluminum capsules, covered with tungstic oxide and enveloped into the capsule. Liquid samples can be handled by means of quartz sample holders (Gács et al., 1977). Furnaces F_1 and F_2 (see Fig. 3) are heated up in manual operation mode, meanwhile combustion chamber B and column D are flushed with oxygen via solenoid valve S , stopcock V_1 and absorber E . At the required temperatures the sample feeding device is filled with samples, the vial transferring device is filled with empty vials, 3-methoxy-propylamine is fed into the carbon dioxide absorber and then the instrument is switched to automatic. The sample preparation cycle is started with a pushbutton. The process can be followed on the flow diagram (see Fig.3) and the operational program (Table 1). As shown in the operational program, a cycle is divided into ten consecutive steps. In the first step the programmer energizes solenoid valve S and actuates the sample feeding device to transfer the sample into the combustion chamber. If

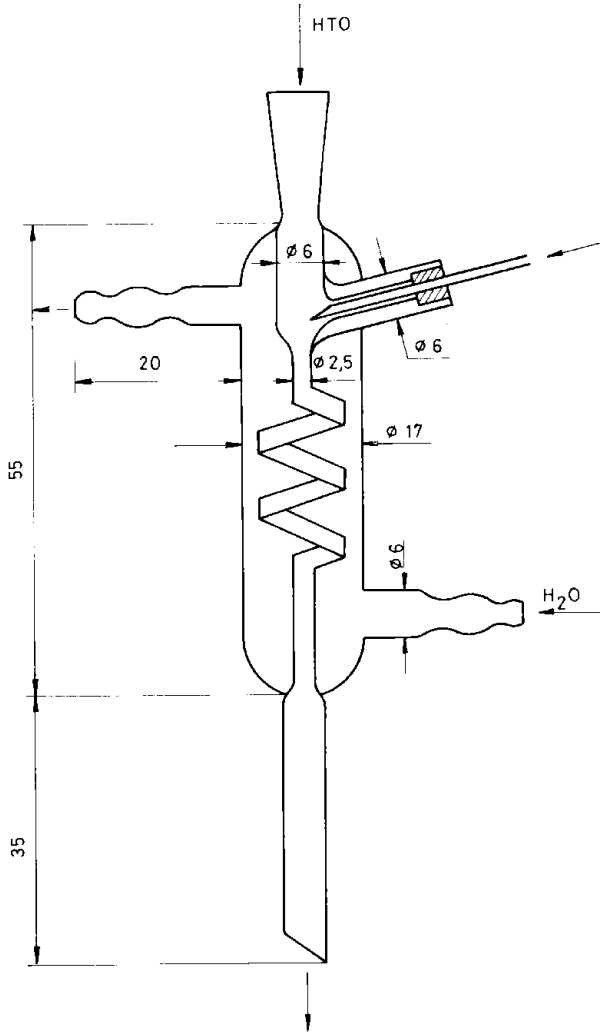


FIGURE 5. Condenser.

the sample exceeds 85 mg, the sample is distributed into 2-3 capsules, which are then burnt consecutively in 30 sec intervals. The combustion products formed are flushed onto alumina column *D* with oxygen stream. The water is retained on the column, while the carbon dioxide is trapped in absorber *E*. In step two stopcock V_2 is turned to open absorber *E* and the radioactive carbonate solution flows into vial G_2 . In step three rinsing of the carbon dioxide absorber with liquid scintilla-

TABLE I. Operational Program

Step No.	Operation	Parts of instrument											Time min.	
		A	S	V ₁	V ₂	H ₁	P ₁	P ₂	P ₃	P ₄	K	M		
1.	Sample in,	X	X	A										2.0
2.	Absorbent out		X	A	X									
3.	Cocktail in		X	A	X				X					
4.	Heating up the column			B	X	X			X					0.1
5.				B	X	X								0.4
6.	Rinsing the column			B		X					X			1.4
7.	Cocktail in		X	B		X	X							0.5
8.			X	B			X							0.2
9.	Change of vials		X	A								X	X	
10.	Absorbent in		X	A				X					X	1.3

X = on, otherwise off

Valve V₁

A = opened to absorber E

B = opened to condenser L

tion solution (fed with pump P₃) is started. In step four solenoid valve S is closed to stop the oxygen stream, stopcock V₁ is turned to connect the column to condenser L and heating up of the alumina filling starts. In step five rinsing of absorber E is completed, therefore pump P₃ is stopped. In step six stopcock V₂ is turned to close the absorber, meanwhile inactive water is fed with pump P₄ into the combustion tube to remove residual tritium. The water eluted on heating the column to 700°C is collected in the condenser and the teflon tube connecting the condenser to vial G₁. In step seven the collected water is rinsed into vial G₁ with liquid scintillation cocktail fed with pump P₁. In this operational step the column is still kept at 700°C. At the same time valve S opens to speed up dehydration of the alumina. In step eight heater H₁ is switched off, meanwhile rinsing of the condenser is still in progress. In step nine stopcock V₁ is turned to connect the combustion chamber to the carbon dioxide absorber, cooling of the alumina filling starts and the vials are changed. In step ten the cooling of the alumina is completed, meanwhile 3-methoxy-propylamine is fed into absorber E with pump P₂. On completion of this last operational step the next sample preparation cycle starts automatically.

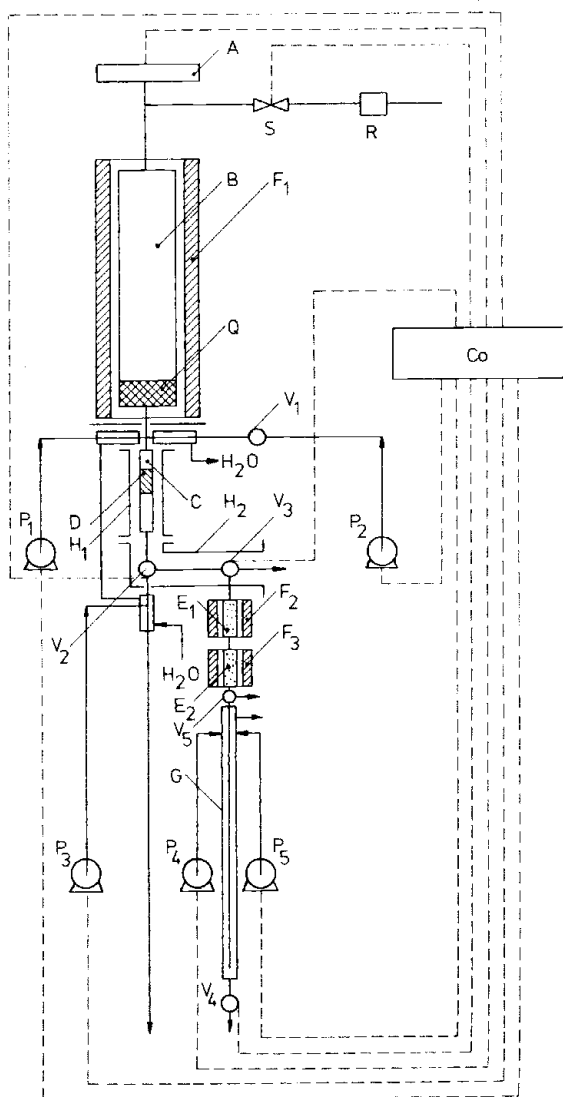


FIGURE 6. Flow diagram of the instrument used for the preparation of samples labelled with carbon-14 and/or sulfur-35

A: sample feeder, B: combustion chamber, C: quartz tube, D: quartz wool column, E_1 : silver wool, E_2 : alumina, F_1 , F_2 and F_3 : furnaces, G: absorber, H_1 and H_2 : heaters, P_1 , P_2 , P_3 , P_4 and P_5 : dispensers, Q: quartz chips, R: flow rate regulator, S: solenoid valve, V_1 : flap valve, V_2 , V_3 , V_4 and V_5 : stopcocks, Co: programmer.

B. Sample Combustion System for Carbon-14 and/or Sulfur-35 Determination

1. *Apparatus.* A schematic diagram of the system is illustrated in Fig.6. Samples weighed into quartz capsules (Gács et al., 1977) are placed into sample transferring device *A*. The sample to be analysed (weight: 1-15 mg corresponding to 0.16-3.2 mg sulfur) is dropped into quartz combustion chamber *B* heated to 1100°C with furnace F_1 . Quartz chips filling *Q* provides a large contact surface during sample combustion in a stream of oxygen. The flow rate of oxygen is adjusted to 80 ml/min with regulator *R*. Sulfur oxides form sulfuric acid in tube *C* on quartz wool column *D* wetted with 4% hydrogen peroxide solution fed in by means of dispenser P_2 (e.g. Type 335A Unipan Scientific Instruments, Warsaw) through a flexible tube which is closed during combustion by means of a flap valve (V_1). Volatile acids formed from nitrogen oxides and halogens are heated off column *D* by electric heater H_1 made from a resistance wire coiled onto a glass tube. At an output of 15 watt this heater gradually warms tube *C* to 100°C.

Three-way glass stopcocks V_2 and V_3 are actuated by small electromotors and kept at 98°C with electric heater H_2 also made from a resistance wire. Halogens are removed from the oxygen stream with silver wool (length: 10 cm) in quartz tube E_1 (length: 10 cm; i.d.: 0.7 cm; o.d.: 1.0 cm) heated to 500°C with furnace F_2 . The water is retained on dehydrated alumina filled into tube E_2 kept at 200°C with furnace F_3 . Carbon dioxide is trapped in absorber *G* containing 3-methoxy-propylamine. The trapping agent is transferred into the absorber through teflon tubing by pump P_4 . The radioactive carbamate solution is washed into a liquid scintillation vial with a toluene based cocktail (5 g PPO and 0.5 g POPOP in 1000 ml toluene) pumped in by means of pump P_5 . Stopcock V_4 is also operated with an electromotor. It opens or closes absorber *G* through which the vial is filled. The sulfuric acid retained on column *D* is rinsed off with distilled water fed in with pump P_1 . The sulfuric acid solution is collected via stopcock V_2 and a condenser. This latter cools the solution before mixing with the liquid scintillation solution (Aerosol MA/toluene system, Szarvas et al., 1971) fed with pump P_3 . Depending on the degree of automation, the liquid scintillation vials can be changed with a vial transferring device (Gács et al., 1978b). Pumps $P_1 - P_5$, stopcocks $V_2 - V_4$, sample feeding device *A* and the vial transferring device (if used) are actuated in due order for a preset time and interval according to an operational program controlled by the programmer (*Co*).

The quartz combustion tube comprising both combustion chamber *B* and tube *C* is shown in Fig.7. The restricted transition part is introduced into furnace F_1 to avoid sulfuric

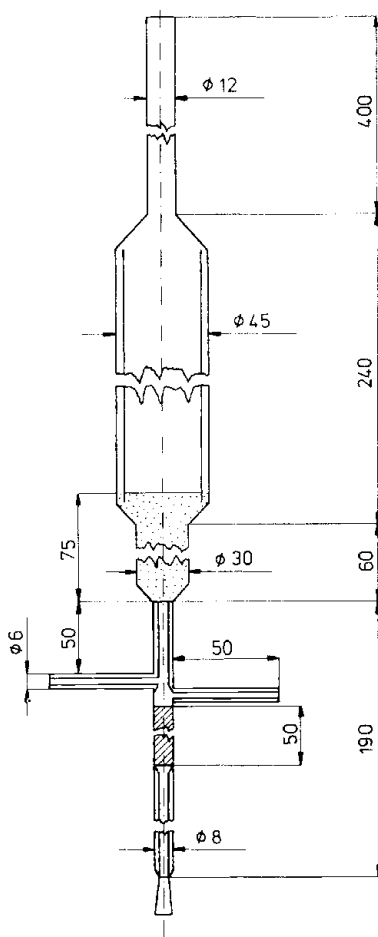


FIGURE 7. Combustion tube for samples labelled with carbon-14 and/or sulfur-35.

acid condensation in this section. The capillary tubes used for delivering water and hydrogen peroxide are fixed into the side arms of tube C with a suitable resin. Small condensers are pulled onto the side arms to provide protection from heat radiation. The heater is self-controlled, with an output that allows tube C to be cooled to ambient temperature (by the water and hydrogen peroxide solution introduced) and then gradually heated to 100°C .

Stopcock V_5 is used in manual operation mode during dehydration of the alumina filling at 700°C in stream of oxygen via solenoid valve S , stopcocks V_2 , V_3 and V_5 (see Fig.6). Application of the alumina filling (E_2) in combination with stopcock V_5 facilitates the use of a carbon dioxide absorber without cooling and also the use of a simple toluene based cocktail containing no emulsifying agent. After heated to 700°C the alumina filling becomes sufficiently dehydrated in a few minutes. Depending on the amount of alumina used and the number of samples analysed this process should be performed at least once a day.

2. *Procedure.* The apparatus is heated up in an oxygen stream. When temperatures of the furnaces (F_1 , F_2 and F_3 in Fig.6) reach 1100°C , 500°C and 200°C , respectively, the sample feeding device is filled up with samples, heaters H_1 and H_2 are turned on, then the sample preparation process, which can be followed on the operational program (Table 2) and the flow diagram (Fig.6) is initiated. Pump P_2 and P_4 are actuated for 20 sec (step one) to transfer hydrogen peroxide solution onto quartz wool filling D , and 3-methoxy-propylamine into absorber G . The excess hydrogen peroxide solution is swept out of tube C by the oxygen stream via stopcock V_2 and V_3 (step two), then the gas stream is directed to absorber G by turning stopcock V_2 (step three). In step four the sample is dropped into the combustion chamber and burnt in stream of oxygen. Carbon dioxide is trapped in absorber G , while sulfur oxides are retained on quartz wool column D gradually heated to 100°C with heater H_1 . Ten minutes after sample introduction only sulfuric acid remains on the column and the quartz wool is apparently dry. In the next step (step five) stopcocks V_2 , V_3 and V_4 are turned and the carbamate solution flows into a scintillation vial. Then the absorber is rinsed with 10 ml liquid scintillation cocktail fed in with pump P_5 , meanwhile the sulfuric acid is rinsed off column D with 5 ml distilled water fed with pump P_1 . Simultaneously, the aqueous sulfuric acid is mixed with liquid scintillation cocktail fed with pump P_3 , and the solution obtained is collected in a vial (step six). In step seven the excess water is removed from the column by the gas stream, and the liquid scintillation solution residue in absorber G is allowed to drip into the vial. After completion of the last operational step (step eight), in which stopcock V_2 is turned to lead the oxygen out of the system via stopcock V_3 closed to the direction of absorber G , the combustion system is ready to start preparation of the next sample. For the radioactivity measurement by liquid scintillation counting calibration established by carbon-14 standards was used, and identical counting efficiency was assumed for sulfur-35.

TABLE II. Operational Program

Step No.	Operation	P_1	P_2	P_3	P_4	P_5	V_2	V_3	V_4	Sample feeder	Time min.
1.	Hydrogen peroxide in, absorbent in		X		X		A	A	C		0.33
2.	Excess hydrogen peroxide out						A	A	C		0.33
3.							A	B	C		
4.	Combustion, evaporation						A	B	C	X	10.00
5.	Absorbent out						B	A	O		
6.	Water in, sulfuric acid out	X		X		X	B	A	O		1.00
7.	Excess water out						B	A	O		0.33
8.							A	A	C		

Pumps : X-on, otherwise off

Valve V_2 : A-opened to V_3 , B-opened to vial

Valve V_3 : A-opened to waste, B-opened to absorber G

Valve V_4 : O-opened, C-closed

III. RESULTS

The procedure developed for the preparation of samples labelled with carbon-14 and/or tritium has been in use in our laboratories and tested by the combustion of labelled organic compounds and biological samples. The results of some of these combustions are collected in Tables 3-6, where \bar{X}_i stands for the individual specific radioactivity values, \bar{X}_i is the mean of the set, and s denotes standard deviation.

Some of the results of isotope analysis of labelled organic compounds used as standard materials are shown in Tables 3 and 4. According to our experiments no memory effect and cross-contamination could be detected. These effects were checked by the combustion of inactive materials after radioactive samples, and by assaying single labelled samples for both isotopes. On the basis of our experimental results obtained by the combustion of standardized organic compounds ad-

TABLE III. Analytical Results

Sample	Weight mg	Radioactivity dpm/mg
Benzoic acid- ³ H	10.188	11566
	14.825	11533
	19.709	11520
	24.450	11626
	31.166	11594
	45.875	11606
	50.165	11679
	70.694	11632
	91.730	11555
	131.320	11469
	160.354	11567
	190.960	11476
	200.788	11542
	212.100	11631
	218.900	11598

$$\bar{X} = 11573 \text{ dpm/mg}$$

$$s = -59$$

$$\frac{s}{\bar{X}} 100 = \pm 0.51 \%$$

ded to different inactive substances of known elemental composition, it may be stated that quantitative analytical recovery is achieved, and neither recovery nor analytical reproducibility is affected by the composition of the sample used.

Analytical results of some series of biological samples from drug metabolic studies are illustrated in Tables 5 and 6. The samples were taken from dried and milled organs. Higher values of deviation in these tables can be attributed to slightly inhomogeneous samples.

Efficiency of the procedure used for the preparation of samples containing carbon-14 and sulfur-35 was tested e.g., by combustion of samples obtained by weighing together standardized benzoic acid-¹⁴C, methionine-³⁵S and inactive substances with halogen, sulfur or phosphorus content. Results of duplicate analyses of some of these samples is shown in Table 7. According to these experiments, in agreement with our previous experience (Gács et al., 1978a), no cross contamination

TABLE IV. Analytical Results

Sample	Weight mg	Radioactivity	
		Carbon-14 dpm/mg	Tritium dpm/mg
1. 1-benzyl-1-(3'-dimethyl- amino-3-propoxy)-cyclohep- tane- ³ H- ¹⁴ C-acidic-fuma- rate	11.548	8972	3553
	20.500	8978	3534
	21.725	8965	3584
	23.590	9071	3564
	30.080	9100	3583
	32.396	8901	3544
	43.000	8980	3602
	57.941	8998	3576
	66.011	9017	3555
	77.131	9023	3570
2. - " -	12.470	16300	497
	35.880	16335	509
	47.005	16214	501
	49.722	16429	486
	70.000	16230	506

$$s_1 = \pm 0.63 \%, \quad ({}^{14}\text{C}), \quad s_1 = \pm 0.58 \%, \quad ({}^3\text{H}),$$

$$s_2 = \pm 0.53 \%, \quad " \quad , \quad s_2 = \pm 1.80 \%, \quad " \quad ,$$

occurs and quantitative recovery of both sulfur-35 and carbon-14 can be achieved.

TABLE V. Results of Duplicate Analysis of Tritiated Rat Tissues^a

Sample	Weight mg	Radioactivity dpm/mg
Kidney	145.000	4831
	140.055	4877
Lung	58.213	1799
	72.614	1823
Brain	119.388	491
	129.099	490
Heart	49.672	1590
	70.422	1568
Spleen	56.911	1663
	69.908	1671
Liver	65.500	5035
	131.577	5100

^aOne single dose of 5 mg/kg 3,4-diacetyl-1-2,5-6-dianhydro-dulcitol-1-³H₁ (50 μCi/mg) was administered i.p. to rats.

TABLE VI. Analytical Results of Dual Labelled Rat Tissues^a

Sample	Weight mg	Radioactivity, (X_i)		$\frac{X_i - \bar{X}}{\bar{X}}$ 100, %	
		¹⁴ C dpm/mg	³ H dpm/mg	¹⁴ C	³ H
Liver	23.805	12143	38922	-0.21	+0.50
	25.300	12115	38611	-0.44	-0.30
	25.704	12223	38976	+0.44	+0.64
	25.961	12084	38519	-0.70	-0.54
	28.350	12282	38612	+0.93	-0.30
Kidney	10.225	4039	69405	-0.88	+0.41
	17.066	4070	69354	-0.10	+0.34
	21.480	4150	68516	+1.84	-0.88
	32.805	4132	69329	+1.40	+0.30
	45.930	3985	69010	-2.21	-0.16
Brain	14.000	502	6174	-3.57	-0.66
	17.227	530	6275	+1.81	+0.97
	24.888	526	6153	+1.04	-1.00
	30.076	539	6266	+3.53	+0.83
	43.133	506	6205	-2.80	-0.21
Spleen	7.114	11569	2575	+0.45	+0.74
	10.805	11589	2578	+0.63	+0.86
	12.966	11339	2536	-1.55	-0.78
	12.983	11750	2585	+2.02	+1.13
	15.898	11518	2508	+0.01	-1.88

^aOne single dose of 20 mg/kg ³,4-(β-phenyl-propionyl)-³,4-³H-1,2,5,6-dianhydro-dulcitol-¹⁴C (545 μCi/mg and 72 μCi/mg, respectively) was administered i.p. to rats.

TABLE VII. Analytical Results^a

Radioactive substance			Inactive substance added		Specific radioactivity	
Sulfur-35					Carbon-14	Sulfur-35
Name	mg	Name	mg	mg	dpm/mg	dpm/mg
Benzoic acid	4.071	Methionine	2.990	S-benzylthiouonium chloride	11596	4355
	1.999	3.065	1.700		11637	4401
	3.306	2.910	2.870	p-Bromo-benzoic acid	11495	4398
	2.551				11517	4332
	3.104	1.014	3.166	o-Iodo-benzoic acid	11600	4305
	3.710				11550	4415
	3.900	2.447	2.405	O-ethyl-S-phenylethyl-phosphorodithioate	11476	4377
	2.684				11563	4350

^a values are corrected for sulfur-35 decay

REFERENCES

- Benakis, A. (1973). "Appareil automatique de combustion d'échantillons contenant du carbone-14 et du tritium comportant un dispositif de collection automatique de $^3\text{H}_2\text{O}$ et de $^{14}\text{CO}_2$ formes". French Patent.
- Gács, I., and Dombi, S. (1978). *J. Radioanalytical Chem.* 42, 375.
- Gács, I., Vargay, Z., and Csetényi, J. (1977). *Mikrochimica Acta* 1977, 107.
- Gács, I., Vargay, Z., and Dombi, S. (1978a). *J. Radioanalytical Chem.* 45, 15.
- Gács, I., Vargay, Z., Dombi, S., Mlinkó, S., Ottinger, J., Prukács, G., Ötvös, L., and Dobis, E. (1978b). "Procedure and Instrument for the Liquid Scintillation Sample Preparation of Materials Labelled with Tritium, or both Tritium and Carbon-14", patent claimed, Hungary, No. MA 3002.
- Gács, I., Vargay, Z., Dobis, E., Dombi, S., Payer, K., Ottinger, J., Prukács, G., and Ötvös, L. (1979). "Procedure and Instrument for the liquid Scintillation Sample Preparation of Materials Labelled with Tritium, or both Tritium and Carbon-14", patent claimed, Hungary, No. MA 3108.
- Kaartinen, N. (1969). *Packard Bulletin* 18.
- Naokes, J. E. (1974). In "Liquid Scintillation Counting, Recent Developments" (P. E. Stanley and B. A. Scoggins, ed.). p. 125. Academic Press, New York.
- Peterson, J. I. (1969). *Anal. Biochem.* 31, 204.
- Peterson, J. I., Wagner, F., Siegel, S., and Nixon, W. (1969). *Anal. Biochem.* 31, 189.
- Szarvas, T., Ömböly, Cs., and Végh, G. (1971). *Radioisotopy* 12, 779.