Advantages of n-Heptanol in the Extraction of 5-Hydroxytryptamine (5-HT)

The quantity of 5-hydroxytryptamine (5-HT, serotonin) present in animal tissues can be conveniently estimated by spectrofluorometry. In the procedure proposed by Bogdansky et al.[1] n-butanol is used as liquor to extract the amine from an homogenate of the tissues at pH 10. This technique gives good results, except when the samples contain comparatively large amounts of 5-hydroxytryptophane (5-HTP), because a certain amount of this substance, which has excitation and fluorescence spectra similar to those of 5-HT, will also be extracted in n-butanol. The problem can be treated quantitatively by calculating the distribution coefficients, K_s, between the alcohol and the buffer phase, of both 5-HT and 5-HTP[2]. By using the values of K_s given in Table I it is possible to calculate the theoretical yields of any given extraction schedule and it is found that, by using the n-butanol technique, it is not possible to determine the 5-HT present in a sample with an error smaller than 15% when the 5-HTP content of the sample is 5 times or more that of the amine. Repeated washing of the organic phase with the buffer reduces the error[3] but also, and considerably, the absolute amount of 5-HT left in the sample, because n-butanol is highly soluble in the aqueous phase (Table I). Thus, the remedy has only limited applications and cannot be used when the total quantity of amine present is 1–2 μg or less.

As a possible alternative many substances were tested to find a substituent for n-butanol. Among these n-heptanol proved fully satisfactory. According to the theoretical yields, calculated as previously explained, it is possible to extract and to measure, with an error smaller than 5%, the 5-HT content of samples containing 50–100 times as much 5-HTP, even when the 5-HT content of the samples is only 1–2 μg. Furthermore, the low solubility of n-heptanol in water permits a more rapid extraction schedule by eliminating all but one washing as well as the final addition of heptane.

As an example of the possibilities of the technique the yields expected and those actually obtained by using samples with known amount of labelled 5-HT and 5-HTP are compared in Table II. The results are satisfactory. The small differences between the expected values and those actually obtained can be explained considering the errors introduced with the empirical determination of the distribution coefficients, which were used in the calculation of the theoretical yields.

The excitation and fluorescence spectra of both 5-HT and 5-HTP in strong HCl are not altered when n-heptanol is used in place of n-butanol, and the technique here reported has been used with good results in spectrofluorometric determinations of the 5-HT content of animal tissues[6].

Zusammenfassung. Mit n-Heptanol als Extraktionsmittel können kleine Mengen von 5-Hydroxytryptamin (5-HT) aus biologischem Material, das 5-Hydroxytryptaphan (5-HTP) in grossen Mengen enthält, extrahiert werden.

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Table I. Comparison of n-butanol and n-heptanol for some characteristics affecting the extraction of 5-HT from biological samples

	n-butanol	n-heptanol
Solubility in water (20 °C), parts per 100, volumes[4]	15.45	0.10
K_s-HT	0.085	0.275
K_s-HTP	9.010	129.000

Table II. Yields obtained with the heptanol technique[5]

5-HT		5-HTP	
Added to sample, μg	Recovered %	Added to sample, μg	Recovered %
0.50	48.0	100	0.048
0.50	52.3	100	0.069
0.50	51.0	100	0.075
1.00	57.7	100	0.075
1.00	52.0	100	0.081
1.00	50.2	100	0.084
1.00	55.2	100	0.087
5.00	49.0	100	0.090
5.00	52.0	100	0.102
5.00	55.2	100	0.108
Average % actual yield ± standard error	52.25 ± 0.94		0.082 ± 0.005
Expected % yield	53.70		0.076
Expected % yield with n-butanol as extractant	71.36		1.090

[1] D. F. Bogdansky, A. Pletscher, B. Brodie, and S. Udenfriend, J. Pharmac. exp. Ther. *117*, 82 (1956).

[2] From the expression:

$$R_s = \left[\frac{K_s V_1}{K_s V_1 + \dfrac{V_2}{n}} \right]^n$$

where V_1 and V_2 are the volumes of the 2 phases; R_s is the fraction of the total amount of S remaining in V_1 after a single extraction and n is the number of extraction steps. Experimentally it is found that, irrespective of the extractant used the value of the ratio K_s-HT/K_s-HTP is a minimum in the pH interval 9.8–10.2.

[3] S. Udenfriend, *Fluorescence Assays in Biology and Medicine* (Academic Press, New York 1962).

[4] *Handbook of Chemistry and Physics* (Chem. Rubber Publications Co., Cleveland 1962).

[5] $C^{14}(2)$-5-HT or $C^{14}(3)$-5-HTP were added to small samples of molluscan ganglia in the amounts given in the Table. The samples were then homogenized in diluted HCl and brought to pH 10 in a final volume of 1.5 ml. 81.0% of the initial homogenate was recovered volumetrically and shaken with 1.5 volumes of n-heptanol. 91.6% of the alcohol was then backwashed with an equal volume of borate buffer, pH 10; after the addition of 0.5 ml ethanol and 15 ml fluorofor the radioactivity of the samples was read in a scintillation counter. n-Heptanol separates rather slowly from the final acidic phase and tends to form a scum at the boundary. Complete separation can be quickly achieved by centrifuging the samples at low speed.

[6] G. A. Cottrell, Comp. Biochem. Physiol. *17*, 891 (1966).

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