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Note

Quantitative elution of thyroidal amino acids from a cation-exchange column: analysis of tablet formulations containing dyes

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The present U.S.P. methods[1–3] for the analyses of sodium liothyronine (T_3Na), sodium levothyroxine (T_4Na) and liotrix (T_3Na–T_4Na combination) tablets are either very lengthy or lack sensitivity and specificity. Although a simple and sensitive method for the analysis of T_3Na has been reported[4] from this laboratory, this procedure is not universally applicable to all brands of T_4Na or liotrix tablets because of the presence of different excipients and dyes. Moreover, an initial separation of the dyes from the active ingredients is advantageous as (1) it eliminates the likelihood of co-elution of the desired amino acid or internal standard with dyes and/or excipients on subsequent analysis by high-performance liquid chromatography (HPLC), and (2), if required to enhance the sensitivity of detection, the isolated amino acid could be derivatized for HPLC or gas–liquid chromatography (GLC) without the possibility of interference from dyes or excipients affecting the derivatization procedure.

Thyroidal amino acids are strongly adsorbed to the surface of the glass vessel and to the chromatographic medium[5–7]. Although adsorption to the glass surface can be eliminated by silanization[5], irreversible adsorption to the chromatographic medium is still a serious problem. Previous procedures for isolation and purification of the thyroidal amino acids involved use of either Sephadex gel or ion-exchange resins as chromatographic medium[5,8,9]. Our attempts to purify the amino acids on Sephadex were abandoned because the commonly used eluting system (methanolic ammonia) degraded the gel. As an alternative, cation-exchange resins of the polystyrene type were tried. These resins are chemically very stable to both highly acidic or basic elution systems. Petersen *et al.*[5] have reported the disadvantages of using a cation-exchange column. They experienced up to 45% loss of triiodothyronine (T_3) and thyroxine (T_4) due to adsorption on the resin even when they used a relatively stronger elution system [methanol–12 *M* ammonium hydroxyide (97 :3)]. To overcome this difficulty, they pre-treated the column with diiodothyronine (T_2), which saturated the adsorption sites, thus permitting quantitative recovery of T_3 and T_4. Since saturation of the adsorption sites with T_2 cannot be universally applied, an alternative approach (use of a much stronger eluting system) is described below.

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EXPERIMENTAL

The chosen solvent system consisted of strongly acidic, water-saturated butanol solution. A known volume of a solution containing T_3, T_4 and 3,3',5'-triiodothyronine (reverse triiodothyronine; RT_3) in a solvent system consisting of equal volumes of 0.2 *M* soidum acetate solution (pH adjusted to 2.2) and dimethylformamide was loaded on to a cation-exchange column (Bio-Rad AG 50W-X2; 100–200 mesh; 10 × 1.6 cm column). The column was thoroughly washed with water (20 times the bed volume) and butanol (3 times the bed volume). The amino acids were then eluted with 60 ml of the upper layer of the mixture obtained by shaking 500 ml of butanol with 500 ml of 3 *M* hydrochloric acid. The eluates were evaporated, and the residues were dissolved in 0.2 to 0.5 ml of 3% ammonium hydroxide solution in methanol. Known aliquots were injected on to the HPLC column under the conditions described by Hearn *et al.*[10]. A typical chromatogram is shown in Fig. 1. In addition, a similar volume of the same mixture of T_3, T_4 and RT_3 without prior purification on an ion-exchange column was directly injected on to the HPLC column. The experiment was conducted in duplicate (on two ion-exchange columns).

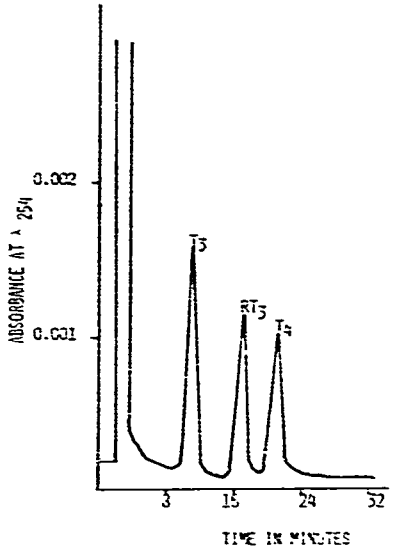

Fig. 1. HPLC chromatogram of a synthetic mixture of *ca.* 0.2 μg each of T_3, RT_3 and T_4 on a μBondapak C_{18} column. The mobile phase was water–methanol–phosphoric acid (50:50:0.1).

Recoveries of T_3, RT_3 and T_4 were calculated by comparing the peak heights for direct injection with those obtained after preliminary separation on the cation-exchange column followed by HPLC. The recoveries were 98–100%; this established that the strongly acidic eluting system quantitatively eluted the amino acids and that the adsorption was reversible.

To demonstrate the applicability of this procedure, a sodium thyroxine tablet (Letter®, 50 μg) containing a pink dye was purified on the cation-exchange column by using the procedure described above and subsequently analyzed on the HPLC column. The resulting chromatogram is shown in Fig. 2.

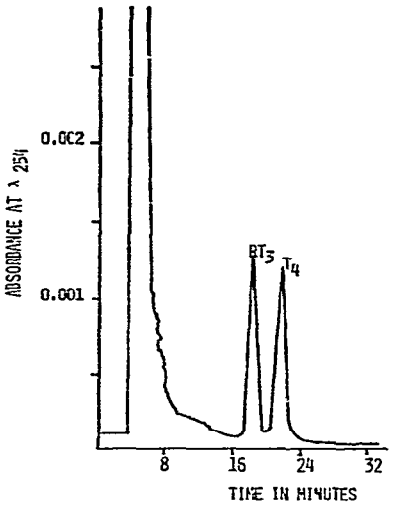

Fig. 2. HPLC chromatogram of sodium thyroxine tablet (Letter®, 50 μg), with RT_3 as internal standard, after purification on Dowex 50 column.

CONCLUSIONS

In conclusion, a procedure is described for the cation-exchange purification of thyroidal amino acids that results in quantitative elution. The procedure has been shown to be useful for the purification of thyroidal formulations containing dyes before analysis by HPLC and it should serve as a general procedure for the isolation and purification of aromatic iodoamino acids from any other matrix when quantitative analysis is desired.

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