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Chromatographical Determination of Radiochemical Purity of Hippuran-^{131}I

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Abstract

A recently known method of paper chromatographical separation of o-iodobenzoic acid-^{131}I and o-iodohippuric acid-^{131}I was found to be in error. The solvent mixture proposed in the method for the efficient separation of the two compounds of similar structure not only be made nonhomogeneous but also brings about no separation. It was also confirmed that no o-iodohippuric acid is converted to o-iodobenzoic acid during the process for Hippuran-^{131}I preparation by isotopic exchange. Upon it, an alternate method of chromatographical determination of radiochemical purity of Hippuran-^{131}I is proposed in present paper.

요 약

페이퍼 크로마토그래피에 의한 o-iodohippuric acid-^{131}I와 o-iodobenzoic acid-^{131}I의 분리에 관한 기보된 방법은 착오인 것으로 밝혀졌다. 이들 두 유사 화합물들의 효과적 분리를 위하여 제시된 용매는 균일하게 섞여지지 않을뿐 아니라 그대로 사용하더라도 이들 두 화합물은 분리되지 않았다.

o-iodohippuric acid-^{131}I의 동위원소 교환법에 의한 제조과정에서 o-iodohippuric acid가 o-iodobenzoic acid로 변환되지 않음을 확인하였으며 Hippuran-^{131}I의 방사화학적 순도 결정을 위한 간편한 방법을 제시하였다.

1. Introduction

Hippuran-^{131}I (abbrev. O* IH) is nowadays widely used for the kidney function studies. In both nephrography and the determination of renal clearance all authors emphasized the radiochemical purity of the product[1-6]. When the contents of the unbound iodide-^{131}I and other impurities are significant they give lower clearance values.

By using paper chromatographic technique, Anghileri[7], Hosick *et al.*[8] and Brown *et al.*[9] found a spot originating from some unkown substance akin to Hippuran, but they failed in identification.

László Varga *et al.*[10,11] reported in 1967 that they firstly identified the unknown compound to be o-iodobenzoic acid-^{131}I (abbrev O*IB) by paper chromatography using benzene: acetic acid;water (2:2:1) as the

developing solvent. However, early in 1964, Walter Hartrodt[12] had already identified the impurity in O*IH to be O*IB with definite Rf value in the chromatography using n-butanol: 2M acetic acid(1:1) as the developing solvent. Mani, R. S. and R. J. V. Prabakaran[13] also reported in 1965 that the impurity is O*IB, and they showed the definite Rf values in the paper chromatography. O*IB may certainly be an impurity in O*IH because it is an intermediate in the commercial production of OIH[14].

When examining the radiochemical purity of O*IH of our product and the imported commercial product according to the method described in the literatures[10, 11] we found that the radiochemical purities of both of ours and the commercial product are only 2 to 3%, and the compounds are mostly O*IB. In this paper the authors point out the faults and differences in the literatures, and propose alternate easy determination method.

2. Experimental

O*IH was prepared according to the method described in the literature[15]. The OIH was purchased from the Mallinckrodt Co., U. S. A. The melting point of OIH (in acid form) was found to range from 170 to 173°C, which is consistent with that in the literatures[10-12]. The concentration of the product, O*IH was ca. 1 mg/ml. and its radiochemical concentration was ca. 800 uCi/ml. O*IB was prepared in the similar way to that applied for the preparation of O*IH. The melting point of OIB was found to be 162°C, and the radiochemical concentration of O*IB was ca. 500 uCi/ml.

One dimensional paper chromatography was performed for the following mixtures;

A; O*IH+*I^-+trace *IO_3 and *IO_4

B; O*IB+*I^-+trace *IO_3 and *IO_4

C; O*IH+O*IB+ trace *IO_3 and *IO_4

D; O*IH+glycine

Whatman No. 1 filter paper and various solvent mixtures (Table 1) were used. The radio paper chromatostrips were dried at room temperature and subsequently scanned by automatic chromatogram scanner (Packard Model 7201). The size of the areas beneath the curves were expressed as the percentage of the applied activity.

To ascertain whether the radioactivity comes out of O*IH or not, the dried chromatostrips were also put under the U. V. light[16] (U. V. lamp, Lab. model for long wave 3660 Å unit).

Various possible impurities were identified by radiochromatogram scanning or sensitive color reaction. Details are given in Table 2.

O*IH reaction mixtures resulted from different reaction time were also sampled for chromatographical ananlysis using solvent mixture of benzene:acetic acid: water (2:2: 0.5) and the upper layer of the solvent mixture of benzene:acetic acid:water (2:2:1).

On the other hand, one dimensional thin layer chromatography were also carried out using Silica gel G plate(Eastman Kodak 6061) and various solvent mixtures listed in Table 3. The T. L. C. plates of 2cm×12cm size were dipped in the solvent mixture contained in small wide mouth bottle (dia. 5cm, height 12cm).

3. Results and Discussion

In the examination of the mixtures of A, B and C using the solvent systems of 1 in Table 1, the Rf value for O*IH is 0.83, and that for O*IB is 0.86, respectively(Table 1). These values agree fairly well with those of the literatures[7, 10, 11, 18]. The solvent mixture of benzene: acetic acid: water (2: 2:1) which is the recommended one in the pharmacopoeia of the U. S.[19] and cited in

the literatures[10, 11] could not be mixed homogeneous if acetic acid volume were not increased twice or if water volume were not decreased half. By modification, we made two kinds of solvent mixtures as shown in Table 1, 2 and 3. Even by using these solvent mixtures the O*IH and O*IB were not separated resulting the Rf value of 0.87 for both O*IH and O*IB (Fig. 1).

However, László Varga *et al.* [10, 11] reported that the solvent mixture of benzene: acetic acid: water(2:2:1) is the most effective one for separation of O*IH and O*IB getting the Rf values of 0.48—0.52 for O*IH, and 0.88—0.92 for O*IB, respectively.

As shown in Table 1, the same Rf values were obtained regardless the methods of developing. Further, we could find that the Rf values are not much different even if the ratio of the solvent component is slightly altered. Thereupon, we emphasize that the solvent mixture of benzene:acetic acid:water (2:2:1) was not made homogeneous, and further it did not bring about the separation of OIH and OIB.

As shown in Table 1, the Rf value of 0.50—0.55 is for $*I^-$. This value nearly coincides with the Rf value of O*IH reported by László *et al.*. The peaks originating from $*I^-$, $*IO_3^-$, and $*IO_4^-$ were identified in comparison with the data obtained by paper chromatography using solvent mixture of methanol: water (3:1)[17] (Fig. 2). The modified solvent mixture of benzene: acetic acid: water(2:2:0.5), and the upper layer of the mixture of benzene:acetic acid: water (2:2:1) are both not effective for separation of O*IH and O*IB but effective for separation of other possible impurities such as $*I^-$, $*IO_3^-$, $*IO_4^-$ etc. Upon it the merit of the complicate manipulation of the chromatography described in the literature[20] is doubtful. We propose that the procedure had better be further revised to the simple description of the solvent mixture or to the alternate method.

According to the data obtained by László Varga *et al.* the Rf value of $*I^-$ and $*IO_3^-$ is both 0.0—0.05, and not separated. However, we obtained different Rf values to give good separation (Fig. 2).

The developed chromatograms were put under the U. V. ray of which the wave length is 3660 Å. In such a way it was confirmed that the most of the radioactivity comes out of the bright spot which is O*IH according to the British Pharmacopoeia[16]. We also tried to confirm the peaks by spraying alcoholic bromophenol blue solution on the chromatostrips developed in benzene: acetic acid: water(2:2:0.5). The color of the spot of O*IH (and OIH) was blue, and that of O*IB (and OIB) was yellow, and it could be clearly distinguished.

Table 1. Solvent mixtures used for development, and Rf values obtained for O*IH, O*IB and $*I^-$

No.	Solvent mixture	Procedure	Rf			Ref.
			$*I^-$	O*IH	O*IB	
1	n-BuOH:HAc:H_2O(4:1:1)	Ascending	0.20	0.83	0.86	(7)(10)(11)(18)
2	Benzene:HAc:H_2O(2:2:0.5)	Ascending	0.53	0.87	0.87	
	〃 〃	Descending	0.55	0.87	0.87	
3	Benzene:HAc:H_2O(2:4:1)	Ascending	0.55	0.86	0.87	
	〃 〃	Descending	0.55	0.87	0.87	
4	Upper layer of the mixture of Benzene:HAc:H_2O(2:2:1)	Ascending	0.55	0.87	0.87	(20)
	〃 〃	Descending	0.55	0.87	0.87	(20)

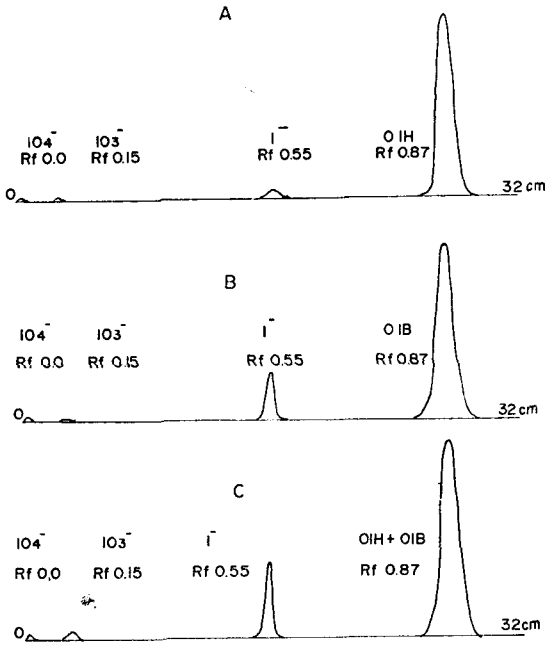


Fig. 1. Chromatogram scan of O*IH, O*IB and *I^-
A shows mixture A, B shows mixture B, C shows mixture C. Solvent mixture; benzene:acetic acid: water (2:2:0.5)

Both spots were quite overlapped showing the Rf value of 0.87.

The results of the paper chromatography for the O*IH reaction mixtures of different reaction time indicate that the peak at the Rf value of 0.87 was increased gradually

Table 2. Rf values of the compounds used for O*IH synthesis# (solvent mixture: No. 2, Table 1)

Compounds	Rf	Methods of detection
Na*I	0.55	Radiochromatogram scanning
Na^*IO_3	00.3—0.16	//
Na^*IO_4	0.00	//
O*IH	0.87	//
O*IB	0.87	//
Glycine	0.14	Alcoholic ninhydrine solution spray

the same Rf values were obtained by using the solvent mixture of No. 4, Table 1.
the Rf value obtained by using the solvent mixture of No. 1, Table 1.

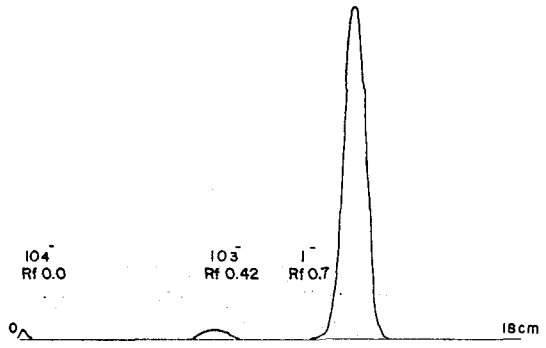


Fig. 2. Chromatogram scan of Na*I solvent mixture; methanol:water (3:1)

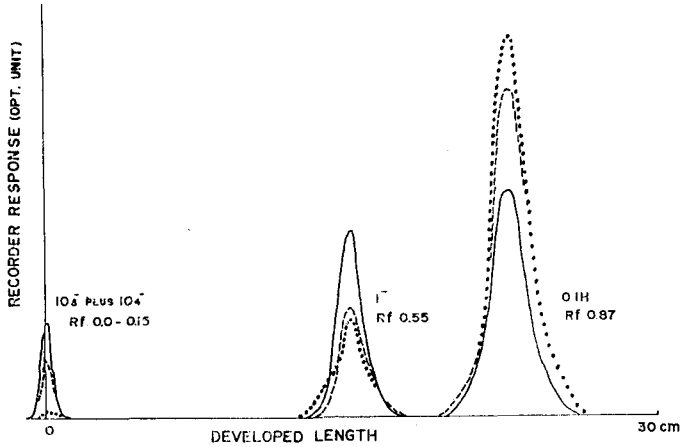


Fig. 3. Chromatogram scan for O*IH reaction mixture of different reaction time (developing solvent, benzene; HAc: H_2O(2:2:0.5))
— : after 30 min., -- : after 90 min., ··· : after 240 min.

Table 3. Solvent mixtures used in the thin layer chromatography of hippuran $-^{131}I$

Solvent mixtures	Rf				
	*I	$*IO_3$	IO_4	O*IH	O*IB
Pet. ether:ethylether (9:1)	0.0				
Acetic acid: water (3:97)	0.0—0.2	0.4	0.0—0.2	0.72	0.72
Ethanol: water:25% aq. ammonia(100:12:16)	0.38	0.0	0.0	0.8	0.8

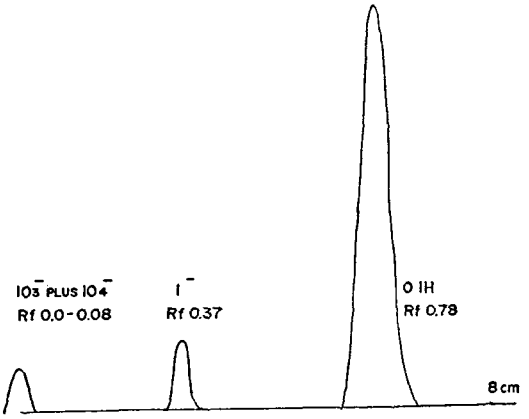
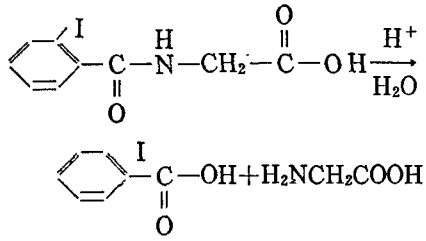


Fig. 4. Thin layer chromatogram scan of crude O*IH product developing solvent mixture; EtOH:H_2O:25% aq. ammonia (100:12:16) developing time; 20 min.

while the peak at the Rf value of 0.55 was decreased gradually (Fig. 3). It also means that the peak at Rf 0.87 originates from O*IH, and that of Rf 0.55 originates not from O*IH but from $*I^-$.

Even though the separation of O*IH and O*IB by T. L. C. failed, the separations of $*I^-$, $*IO_3^-$ and O*IH were successful (Table 3 and Fig. 4). The separations were clearly accomplished in 20 minutes. In so far as the raw material, OIH, is relatively pure, the SiO_2 gel T. L. C. using the solvent mixture of ethanol: water:25% aq. ammonia(100:12:16) may be the best method since there is no reson for the presence of O*IB. During the exchange reaction O*IB is not formed by acid hydrolysis as following;



In the chromatography of the reaction mixture of O*IH no glycine was detected under ninhydrine spray. The Rf of glycine was 0.14 (Table 2).

4. Conclusion

Previously known method of paper chromatographical separation of o-iodobenzoic acid-^{131}I and o-iodohippuric acid-^{131}I was found to be in error. Only free iodide-^{131}I, iodate-^{131}I and o-iodohippuric acid-^{131}I were separated by using similar solvent mixture of benzene: acetic acid: water (2:2:0.5).

The solvent mixture of benzene: acetic acid: water (2:2:1) could not be made homogeneous. For rapid routine check of radiochemical purity of Hippuran-^{131}I, T. L. C. technique using silica gel G plate and a solvent mixture of ethanol: water: 25% aq. ammonia (100:12:16) is preferable under the circumstances of pure cold Hippuran is used for the preparation of Hippuran-^{131}I.

References

1) Mescham I. *et al.*, J. Nuc. Med. **4**, 70 (1963)
2) Farmer, C.D. *et al.*, *ibid.*, **6**, 532 (1965)
3) Blaufox, M.D. *et al.*, *ibid.*, **4**, 423 (1965)
4) Greenlaw, R.H. *et al ibid.*, **5**, 453 (1964)
5) Burbank, M.K. *et al.*, Proc. Staff. Mtgs, Mayo clinic **36**, 372 (1961)
6) Tauxe, W. N. *et al.*, *ibid.*, **39**, 761 (1964)
7) Anghileri, L.J., J. Nuc. Med., **4**, 155(1963)
8) Hosick, T. A. *et al.*, *ibid.*, **5**, 551 (1964)
9) Brown, D.W. *et al.*, *ibid.*, **6** 287 (1965)
10) László Varga *et al.*, *ibid.*, **9**, 604 (1967)
11) Varga László *et al.*, Acta Pharn. Hungaria **38**,

15 (1968)
12) Walter Hartrodt, J. Nuc. Med., **4**, 423 (1965)
13) Mani, R.S. and R.J.V. Prabakaran, Curr. Sci. (Bangalore) **35**, 16 (1966)
14) Otakar Charamza, Jiri Opavsky, Unitr. Lek,. **11, 12**, 1211 (1965)
15) J. Kim, J. Korean Nuc. Soc., **4**(3), 186(1968)
16) British Pharmacopoeia 918-919 (I968)
17) United States Pharmacopoeia **XV**, 662 (1955)
18) Tubis, M. *et al.*, J. Nuc. Med. **5**, 532 (1964)
19) United States Pharmacopoeia **XVII**, 618 (1964)
20) United States Pharmacopoeia **XVIII**, 639(1970)