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IP-3 Labelling of S(-)BZM with Iodine-125 using Chloramine-T and Iodogen as Oxidizing Agents.

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ABSTRACT

Labelling of (S)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-hydroxy-3-iodo-6-methoxy benzamide [S(-)-BZM] with iodine-125 using chloramine-T and iodogen as oxidizing agents was studied. The labelling yield was highly dependent on the pH of the reaction medium, S(-)BZM concentration, amounts of oxidizing agents and on the reaction time. High labelling yield greater than 90 % was obtained by reacting 0.24 μM S(-)BZM solution with 0.24 μM chloramine-T solution in phosphate buffer of pH 3 at room temperature for not more than 3 min. When iodogen was used as oxidizing agent , the labelling yield was found ≥ 80 % under the same conditions mentioned earlier. The advantages of the use of iodogen as oxidizing agent are : its molar ratio to substrate does not has a great effect on the percent yield, no side products were produced as a result of the prolongation of the reaction time, and finally it is easy to be removed from the reaction mixture.

Key words : S(-)BZM / Chloramine-T / Iodogen coated glass tubes / Iodine-125 labelling / HPLChromatography .

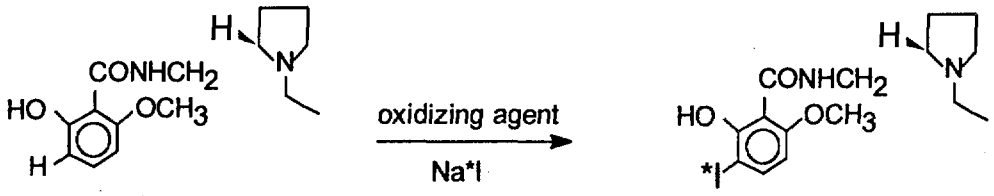
INTRODUCTION

(S)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-hydroxy-3-iodo -6-methoxy benzamide ([^{123}I] IBZM) is central nervous system (CNS) D_2 dopamine receptor imaging agent, belongs to a group of structully related benzamides which display significant antidopaminergic activity [1-3]. The pharmacological effect of S(-)BZM assumed to be induced by blocking the central nervous system D_2 receptor with high affinity and stereospecificity [4,5]. Radioiodinated benzamide was prepared at no-carrier added level. Preparation of the no-carrier added ^{125}I-IBZM has been achieved by an oxidative iodination of S(-)BZM with sodium ^{125}I iodied as shown in figure 1. In this reaction the electrophilic radioiodine can be generated by a varity of oxidizing agents such as chloramine-T, hydrogen peroxide, sodium persulfate, m-chloroperoxybenzoic acid and peracetic acid [6]. Mei-ping Kung and his co-workers stated that, of all the oxidizing agents tested, peracetic acid appear to be the best agent for no-carrier added radioiodination. The use of peracetic acid produces high radiochemical yield and purity with short reaction time.

Recently, Hu-Mingyang et al[7] synthesize and labelled S(-)BZM with radioiodine using H_2O_2 as an oxidizing agent and at room temperature with labeling yield > 80% and radiochemical purity > 90%.

Chloramine -T is commonly used as oxidizing agent for labelling of proteins and small molecules labelling with radioactive iodine. Due to its strong oxidation potential, this oxidant is prone to producing side reactions in addition to chlorinated

side products which decrease radiolabelling yield and are difficult to separate from the desired iodinated compounds [8]. To prevent the formation of both side and chlorinated products, labeling of S(-)BZM with radioiodine using iodogen in comparison with chloramine-T was studied. Factor affecting the radiochemical yield such as pH of the reaction mixture, reaction time, concentration of S(-)BZM, concentration of the oxidizing agent, and amount of KI carrier were studied. Product stability as function of time was also studied.



S(-)BZM *IBZM

Fig. (1) : Oxidative iodination reaction of (S) - N - [(1-ethyl - 2 pyrolidinyl) methyl] - 2-hydroxy-3-iodo-6-methoxy benzamide.

EXPERIMENTAL

Materials

(S)-N-[(1-ethyl-2-pyrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxy benzamideS(-)-BZM : was purchased from RBI (Research Biochemical International, USA), Chloramine-T (Aldrich) and 1,3,4,6-tetrachloro-3α,3α diphenylglucoril, ,Iodogen, (Pierce Chem. Co.) were used without further purification. ^{125}I is no-carrier-added (MDS Nordion S.A., Belgium). All other reagents and solvents used were of analytical grade.

Labelling techniques:

The radioiodination of S(-)BZM was achieved by two methods:

Chloramine-T method:

In a reaction vial containing an appropriate amount of the substrate S(-)BZM in ethanol, a suitable amount of radioiodide (10 μl, 50μCi) and 300 μl of sodium phosphate buffer pH 3 or ammonium acetate buffer pH 4 were added followed by the addition of chloramine-T solution of the desired concentration in a total reaction volume of 450 μl. The reaction is allowed to proceed for a chosen interval of time, after which the reaction was terminated by the addition of sodium metabisulphite (100 μl, 200 mg/ml) and neutralized with saturated sodium bicarbonate (500μl).

Iodogen method :

Five milligrams of iodogen were dissolved in 5 ml of chloroform and a volume containing a desired amount of iodogen is placed in the reaction vial, the solvent is allowed to evaporate forming a thin film on the wall of the reaction vial. To this coated vial, a solution of the substrate and radioiodide were added and the volume was completed to 450 μl. After a

chosen interval of time the reaction is stopped by removing the reaction mixture from the reaction vial and neutralized with saturated sodium bicarbonate (500 µl).

Radiochemical yield determination :

The product was analysed directly by TLC (silica gel 60, MN Duran, Germany) using the solvent system CH_3Cl : C_2H_5OH : conc. NH_3 (9:1:0.2). The free iodide stayed at the origin with $R_f = 0$, whereas iodobenzamide migrate with $R_f = 0.8 - 0.9$. The purity study using high pressure liquid chromatography, HPLC, was performed on the reversed phase Lichrosorb RP-18 column (250 x 4mm) eluted isocratically with the solvent system : acetonitrile : 10 mM 3,3- dimethyl glutaric acid, pH 7 (82:18) at flow rate of 1ml/min. The radiochemical yield of ^{125}I-IBZM was calculated as the ratio of the radioactivity of the labelled product to the total activity. The reported yields are the mean value of three experiments.

RESULTS AND DISCUSSION

Preparation of radioactive ^{125}I-IBZM by radioiodination reaction was studied using chloramine-T and iodogen coated glass tubes as oxidizing agents. The labelled product obtained was compared with chemically pure non-radioactive IBZM on HPLC using RP-18 column. Based on the elution profiles, it is clearly demonstrated that the radioiodinated IBZM display the same retention time ≈ 20 min as that of the authentic sample (cold IBZM detected by U.V. tracing). While the noniodinated starting material, S(-)BZM , under the same chromatographic conditions, showed a retention time of 20 min. HPLC radio- chromatogram was shown in Fig. 2. When chloramine-T and iodogen used as oxidizing agents, the radioiodinated product displayed high radiochemical purity ≥ 90 % and 80 % respectively.

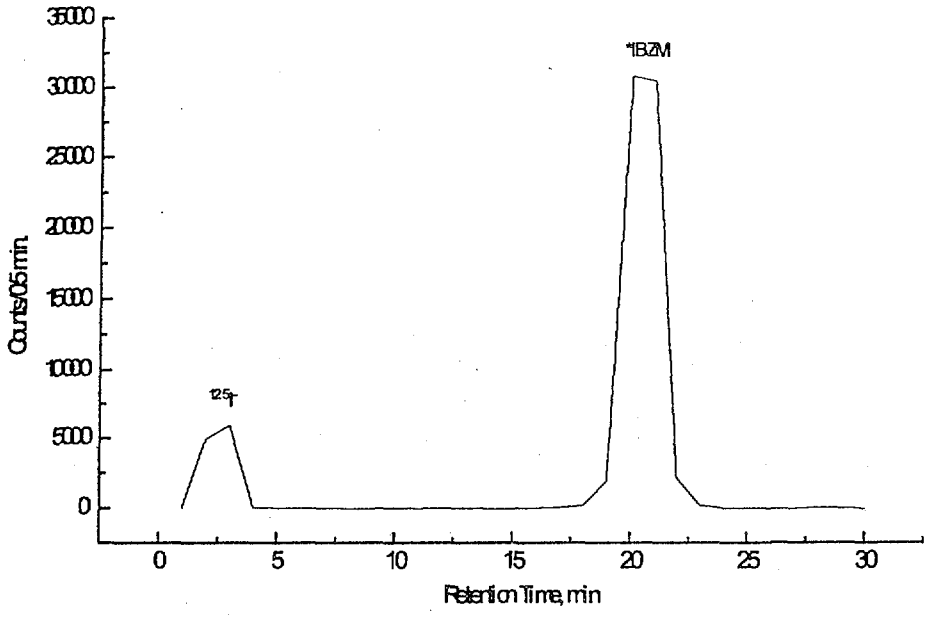


Fig. (2) : UV and radiochromatogram of ^{125}I-IBZM.
Elution conditions : (RP-18 column, the elute solvent is acetonitrile : 10 mM 3,3- dimethyl glutaric acid, pH 7 (82:18) at flow rate 1ml / min)

Influence of pH of the reaction mixture :

The effect of pH of the reaction medium on the labelling of S(-)BZM with ^{125}I using chloramine-T or iodogen was investigated in pH range from 1 to 12. Fig. 3 shows that the maximum yield was attained at pHs 3 and 4.

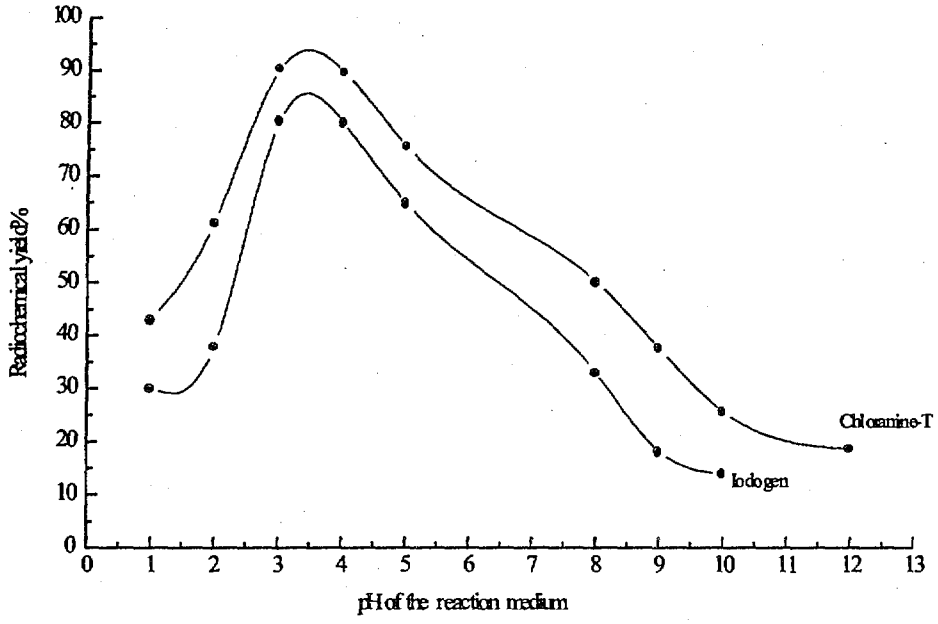


Fig. (3) : Effect of pH of the reaction mixture on the labelling yield of S(-)BSM with iodine-125 using chloramine-T or iodogen as oxidizing agents.

Reaction conditions: (0.24 μM S(-)BZM, 0.24 μM CAT in different buffer systems with appropriate pH values at room temperature and reaction time 3 min)

Influence of reaction time:

Labelling of benzamide with iodine-125 was carried out in 0.5 M phosphate buffer pH 3 using both oxidizing agents chloramine-T and iodogen. The molar ratio of chloramine-T to substrate used is (1 : 1). The labelling yield was determined at different time intervals using HPLC system. The results of this study was presented in Fig. 4 and clearly shows that , the labelling of S(-)BZM with CAT in acidic pH leading to produce high yield ≥ 90 %, when the reaction time is 3 min. But when the reaction was allowed to proceed for more than 3 min the yield was not increased. The yield of ^{125}I-IBZM was found ≥ 80 % and complete within 9 min when iodogen coated glass tube was used. The reaction in case of chloramine-T is faster and high than that of iodogen because of the presence of CAT in solution whereas iodogen react on the reaction vial boundaries causing less contact with the components of the reaction mixture.

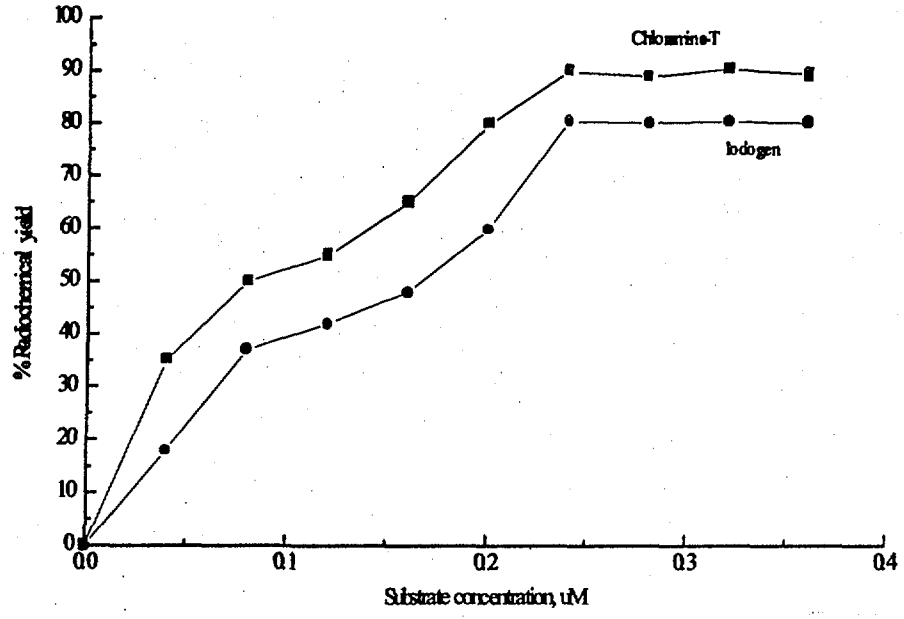


Fig. (5) : Effect of S(-)BZM concentration on its percent labelling with iodine-125

Reaction conditions: (x M S(-)BZM, 0.24 μM CAT or iodogen, KI in 0.5 M phosphate buffer pH 3 at room temperature and reaction time 3 min)

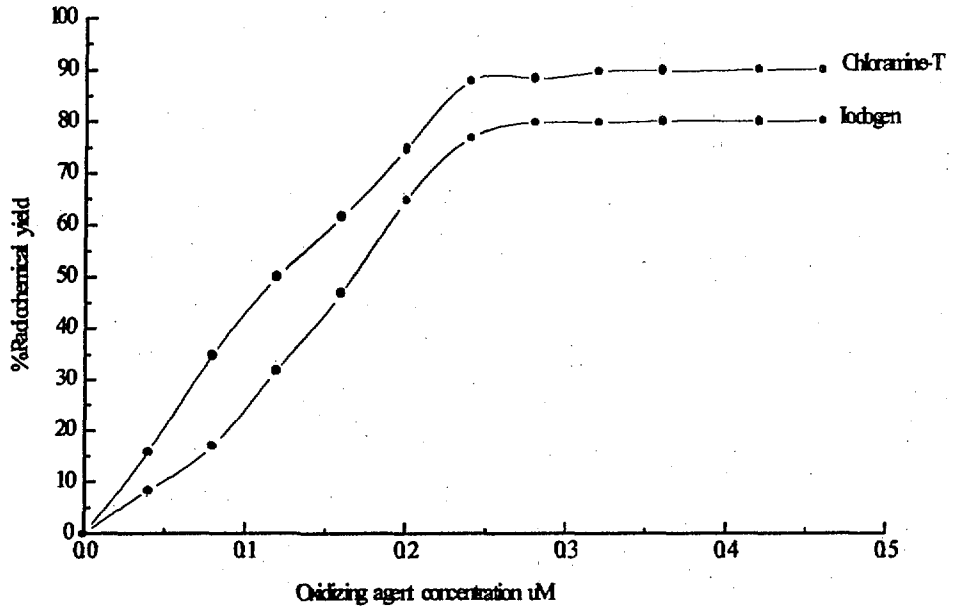


Fig. (6) : Effect of oxidizing agents concentrations on the percent labelling yield of S(-)BZM with iodine-125.

Reaction conditions: (0.24 μM S(-)BZM, 0.24 μM CAT or iodogen, KI in 0.5 M phosphate buffer pH 3 at room temperature and reaction time 3 min)

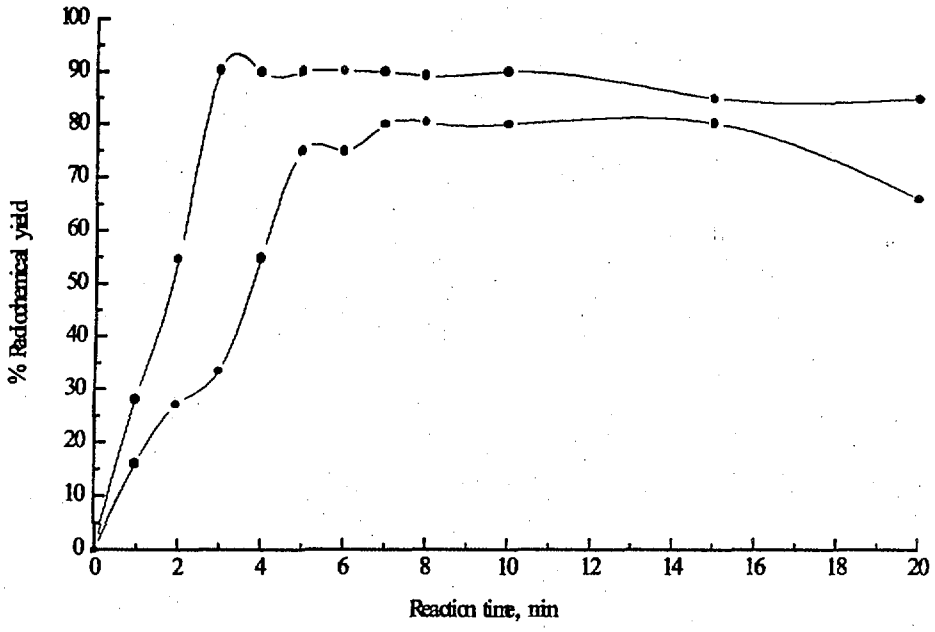


Fig.(4) Variation of the radiochemical yield of ^{125}I-IBZM with reaction time.
Reaction conditions: (0.24 μM S(-)BZM, 0.24 μM CAT iodogen, KI in 0.5 M phosphate buffer pH 3 at room temperature and different reaction time)

Substrate concentration dependence :

Fig. 5 shows the variation of the labelling yield of ^{125}I-IBZM with the concentration of substrate at constant CAT or iodogen concentrations. Data indicate that the yield of ^{125}I-IBZM increases with the increase in S(-)BZM concentration at low values (0.05 0.24 μM) and further increase in substrate concentration has no significant effect on the yield. It is found that the concentration of 0.24 μM of substrate is sufficient to reach a maximum yield of about 90%. This indicates that the substrate is highly reactive towards the electrophilic substitution reactions and this due to the presence of phenolic OH^- [9]. As expected for a second order reaction, the iodination reaction occurred faster with higher substrate concentration and the 0.24 μM of substrate was chosen and kept constant in all other experiments.

Dependence of the yield of ^{125}I-IBZM on oxidizing agents concentration :

The effect of the variation in the concentration of chloramine-T or iodogen on the labelling yield of ^{125}I-IBZM was investigated. Results in Fig.(6) indicate that an increase in the oxidizing agents concentration increase the radiochemical yield to a maximum value (90% or 80%) and further increase has no effect on the yield. Also, It was found that increasing the molar ratio of chloramine-T : S(-) BZM from 1:1 to 10 :1 leads to decrease the radiochemical yield of [^{125}I]IBZM from ~ 90 % to ~ 60 % as shown in table 1. But the molar ratio of substrate : iodogen has no effect on the percent labelling yield.

Table (1): Effect of oxidant : substrate molar ratio on the radiochemical yield percent of ^{125}I-IBZM

Chloramine-T		Iodogen	
Molar ratio	Yield %	Molar ratio	Yield %
1:1	90	1:1	80
2:1	85	2:1	78
4:1	71	4:1	79.8
8:1	67	8:1	80..5
10:1	61	10:1	80

KI carrier dependence:

Various amounts of KI carrier were mixed with Na^{125}I and added to the reaction mixture containing 0.24 μM of S(-)BZM and 0.24 μM of the chloramine-T. At these concentrations the labelling yield is about 90%. As shown in Figure 7 the labelling yield in case of chloramine-T and iodogen decreased with the increase of KI concentration. Petzol, G. et al [10] reported that the decrease in the radiochemical yield with the increase in KI carrier concentration is due to a competition between the radioactive and inactive iodide.

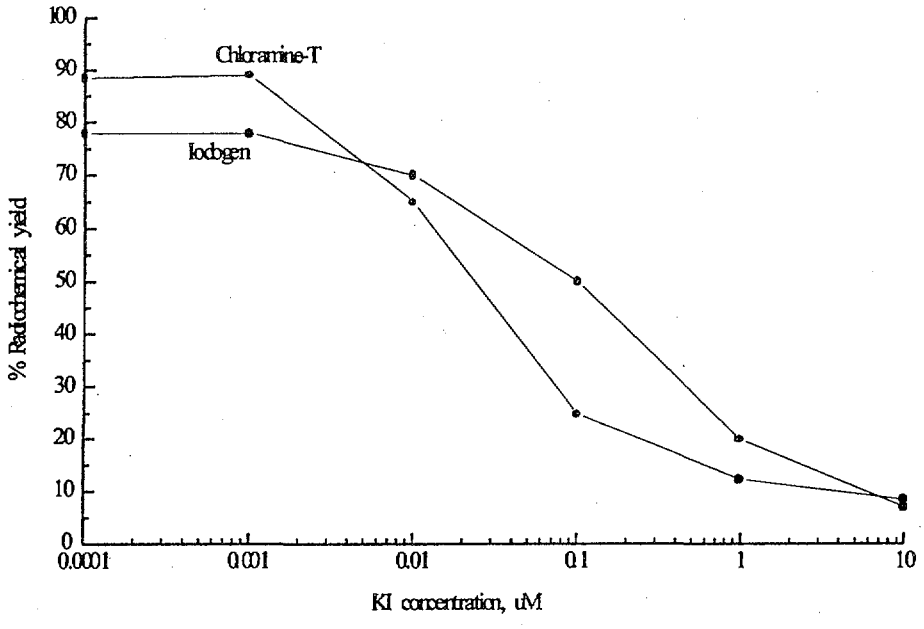


Fig. (7) : Effect of KI carrier on the percent labelling yield of S(-)BZM with iodine-125.
Reaction conditions: (0.24 μM S(-)BZM, 0.24 μM CAT or iodogen, x KI in 0.5 M phosphate buffer pH 3 at room temperature and reaction time 3 min)

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