A RAPID METHOD FOR THE SYNTHESIS OF 4-IODOANTIPYRINE

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Summary: A modified method for the synthesis of radioiodinated 4-^{125}iodoantipyrine based on electrophilic iodination using $NaIO_3$ as an oxidising agent is described. This straightforward method is reproducible and yields consistently high (>98%) radiolabelled 4^{125}IAP. The use of solid phase extraction columns facilitates the removal of excess iodide and chloride ions with consistently high recovery (>80%), simplifies the purification procedure, reduces preparation time and does not require the use of hplc technique.

Keywords: 4-iodoantipyrine, sodium iodate, high performance liquid chromatography, solid phase extraction, thin layer chromatography, radioiodination.

INTRODUCTION

Antipyrine (AP) 1,5-dimethyl-2-phenyl-3-pyrazolone has analgesic and antipyretic properties and is used in veterinary medicine for horses(1). The iodinated analogue of AP, 4-Iodoantipyrine (4IAP) is lipophilic, readily crosses the intact blood brain barrier and

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the regional delivery is flow limited. 4IAP has been used to study regional cerebral blood flow and to detect avascular space occupying defects(2). Although the positron emitting radionuclide ^{11}C, is suitable for PET scanning, ^{11}C labelling of AP has built-in disadvantages such as the short half life ($t_{1/2}$= 20.4min.), the relatively complicated and time consuming synthetic procedure, and requires expensive on-site irradiation and shielding facilities(3). In contrast, radiolabelling with iodine is much more convenient. ^{123}I ($t_{1/2}$=13hrs.) labelled 4IAP can be used for obtaining SPECT images, ^{124}I ($t_{1/2}$= 4days) 4IAP for studying pharmacokinetics and other physiological processess using the PET technique and ^{125}I ($t_{1/2}$= 60days) 4IAP for tumour blood flow study in animals. Various methods are published describing different techniques of radioiodination; radioisotopic exchange with 4-bromoantipyrine and 4-iodoantipyrine(4,5), silica surface catalysed iodination(6,7), use of chloramine T as an oxidant(8) and isotope exchange by the melt technique(9).

This work details the radioiodination of AP using $NaIO_3$ as an oxidising agent for the rapid preparation of 4-^{125}IAP and subsequent purification by reverse phase, high performance liquid chromatography (RP-HPLC) or solid phase extraction (SPE) technique. The process takes less than one hour and consistently high radiolabelling with high product recovery is achieved. The technique described using SPE is reproducible, simple and can be undertaken without highly trained staff and and does not require HPLC.

MATERIALS AND METHODS

1,5-dimethyl-2-phenyl-3-pyrazolone, commonly known as antipyrine (AP) was obtained from Sigma-Aldrich Chemicals Co.. Sodium iodide (NaI), sodium iodate($NaIO_3$), hydrochloric acid(HCl), ethanol

(C_2H_5OH) and methanol(CH_3OH) were obtained from BDH plc.. All reagents were of AR grade and were used as received. $Na^{125}I$ (100mCi/mL) was supplied by ICN Biomedicals Ltd.. The strong anion exchange (SAX), solid phase extraction (SPE) columns were obtained from Alltech Ltd.(IC-OH Maxiclean cartridge) and 0.22μm sterile filters were supplied by Millipore. Silica gel 60°A MK6F plates supplied by Whatman Ltd. were used for tlc analysis. The identification and separation of 4^{125}IAP were achieved using a Spectra physics SP 8810 system, SP 4290 integrator and Spectra 100 (UV and Visible) variable wavelength detector. A Beckmann 170 radioisotope detector connected in series allowed simultaneous detection of chemical and radiolabelled products independently. The tlc plates were scanned to monitor the distribution of the radioactivity by a Tracemaster 20, automatic tlc analyser supplied by Berthold (U.K.)Ltd..

Preparation

0.1M AP, 0.1M NaI and 0.1M HCl solutions were prepared using ultrapure water. Aqueous solution of $NaIO_3$ containing 1mg $NaIO_3$ (5nmole) were dispensed into plastic reaction vials (eppendorf tubes) and dried in vacuo or evaporated to dryness in a fume cupboard. These dried tubes can be stored at 4°C for future use. The SAX-SPE column (IC-OH Maxiclean cartridge) was prepared by washing with 2mL methanol and then 2mL water prior to use.

Labelling Procedure

30μL 0.1M AP, 10μL $Na^{125}I$, 10μL 0.1M NaI were mixed together in the eppendorf tube containing 1mg $NaIO_3$, followed by 50μL 0.1M HCl and mixed thoroughly. The reaction mixture was placed in a thermostatically controlled hot water bath (80°C) for 2-5

minutes. The resulting solution was drawn into an injection syringe and loaded onto the pretreated Alltech IC-OH, SPE column. The SPE column fits directly onto a luer or luerlock syringe hub facilitating transfer of solution. This gives extra safety and ease of operation. To obtain a sterile product, a 0.22μm sterile filter may be connected in series. The SPE column was washed with 100μL water and the washing was discarded. Then the column was eluted with 3 x 1 mL ethanol. The eluate was collected and measured for radioactivity. The eluate may be dried by using compressed air or nitrogen and reconstituted as required using isotonic saline prior to clinical use or stored in ethanol at 4°C until further use. The labelling procedure must be performed in the radiochemical fume cupboard in accordance with the radiation safety regulations.

Alternatively, purification may be achieved by using the HPLC technique and employing Alphasil 5 OD, C_{18} RP column, 4.8mm x 250mm (HPLC Technology), with 40:60 v/v methanol:water mixture as an eluent maintaining flow rate at 1mL/min. and monitoring at 254nm. NaI, AP and 4IAP were detected at R_t 2.37, 8.36 and 12.45 min. respectively. The RP-HPLC technique was used for quality control and for verifying results.

Quality Control

The presence of free iodide in the reaction mixture can be checked by tlc technique, using silica gel 60°A MK6F plate and 1:1 v/v toluene:ethylacetate mixture as an eluant; free iodide, AP and 4^{125}IAP were detected at R_f 0.0, 0.26 and 0.55 respectively. The RP-HPLC technique using conditions mentioned as above was used for verifying all experiments. The RP-HPLC results based on total iodine showed radiochemical labelling of

>98%. Using the SPE-SAX column >80% of 4^{125}IAP was consistently recovered.

Purification

Using RP-HPLC technique and the conditions described above, the 4^{125}IAP fraction may be collected for use. However, using the SPE-SAX (Alltech IC-OH) column, unreacted radioactive iodide and excess chloride ions can be removed and contained within the column, reducing the risk of radioactive contamination and also reducing the acidity of the solution from pH 1 to pH 7. It is noteworthy that the eluate from SPE-SAX column containing unlabelled AP was used for tumour blood flow studies in animals and no adverse physiological reactions were observed. Three solvents were tried in order to remove excess AP by solvent extraction technique; diethyl ether was found to be more efficient than chloroform or dichloromethane.

RESULTS AND DISCUSSION

The series of preliminary experiments indicated that it is possible to prepare all reagents in 0.1M HCl and achieve similar radiolabelling avoiding addition of 0.1M HCl, except that NaI solution slowly turns straw yellow in light producing I_2, resulting in reduced %labelling. Since low concentration of $Na^{125}I$ was used for the reaction, NaI as a carrier was used to suppress other competing reactions affecting the labelling process(10). The use of $NaIO_4$ instead of $NaIO_3$ as an oxidant resulted in equally high %labelling, but $NaIO_4$ solutions were found to be less stable and delayed separation initiated hydrolytic decomposition of 4IAP. In view of the toxicity of K^+ ions in humans, the use of KI and KIO_3 were avoided. No radioiodination was possible using iodogen as an oxidant(8).

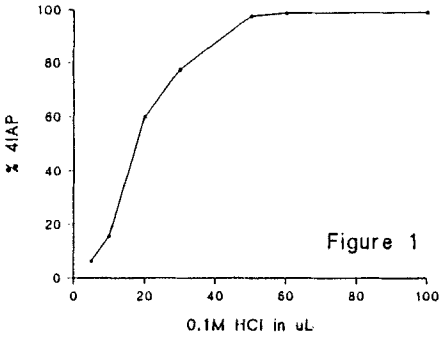

Figure 1

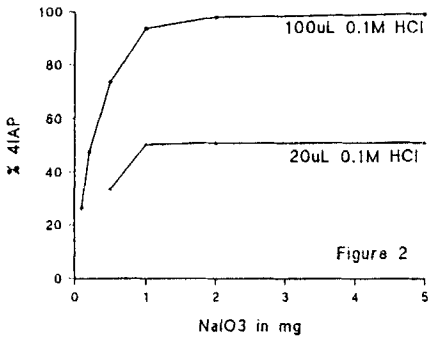

Figure 2

Using the standard conditions described in the labelling procedure and on increasing only the quantity of AP up to 100µL, the labelling efficiency remained unchanged. Similarly, on increasing NaI carrier up to 50uL and $NaIO_3$ upto 5mg the labelling was unaffected. The most important factor was found to be the final pH of the solution and the reproducible and highest yields were obtained for the solutions with pH <2.0, Figure 1(4). It was possible to produce 4^{125}IAP using n.c.a. $Na^{125}I$ provided the minimum volume was 50µL. It was also observed that the addition of excess acid (100µL) did not affect %labelling adversely, but the addition of insufficient acid (20µL) reduced the %labelling (Figure 2). The fact that the use of excess acid (100µL) did not affect labelling efficiency suggests that the process of radioiodinaton occurs through the production of I^+ species(5) and the stability of I^+ in aqueous solution can be improved by increasing acidity(11). However, while studying the effect of oxidant concentration, it was observed that on using 0.1mg (0.5nM) and 0.2mg (1.0nM) $NaIO_3$ the labelling efficiency was low suggesting insufficient oxidant. On standing, the solution changed from clear to straw yellow indicating formation of I_2, but no change in the yield of 4^{125}IAP was observed. Molecular iodine did not react with AP in acidic solution to form 4^{125}IAP, suggesting the possible formation of short lived, active species of iodine produced in the presence of $NaIO_3$ and 0.1M HCl

responsible for radioiodination. However, the labelling efficiency remained unchanged on further increase of $NaIO_3$ up to 5mg (25nmole). The commercial unavailability of $Na^{125}IO_3$ prevented the investigation of the role of IO_3^- ions in the radiolabelling process and the fate of iodine from the IO_3^- ions in the production of 4^{125}IAP was not explored, as this may help to determine whether 4^{125}IAP produced is carrier free or not.

CONCLUSION

This one step synthesis of 4^{125}IAP does not require addition of other reagents to control, neutralise or to buffer the reaction. The maximum labelling occurs when the final pH is less than 2. Using a SAX-SPE column, the removal of unreacted radioiodide ions and excess chloride ions can be achieved in one step and resulting neutral solution containing 4^{125}IAP and AP can be used directly. If necessary, AP can be completely removed by solvent extraction process using diethyl ether. It is thought that the radioiodination occurs through production of I^+ species, which are more stable in the acidic solutions. This method is straightforward, reproducible, quick and yields consistently high (>98%) 4^{125}IAP and high product recovery (>80%).

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