BIODISTRIBUTION AND PHARMACOKINETICS OF I-131 LABELLED 4-IODOPHENYLACETIC ACID.

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Abstract

Phenylacetate has been reported to have a potent anti-proliferative and anti-differentiating effect in haematological malignancies and in solid tumours at non-toxic concentrations. This study is a preliminary investigation of ^{131}I-radiolabelled 4-iodophenylacetic acid as a potential radiopharmaceutical equivalent. Radiolabelling by isotope exchange gave a radiochemical yield of 53 ± 6 %, and a radiochemical purity of 97.8 ± 1.2 %, as qualified by HPLC. The labelled product was used in Sprague Dawley rats and athymic nude (balb/c) mice xenografted with WHCO1 cells (an oesophageal cancer cell line). Dynamic and static scans were carried out on *rats with a SPECT camera to determine the biodistribution of 4-[^{131}I]-iodophenylacetic acid. No target organ was found after 5 h with fast excretion from all organs via the kidney into the urine. *Ex vivo* studies (termination 5 h after injection) were performed in 12 xenograft mice carrying tumours of 5-8 mm on their right flank. Tumour uptake of 4 + 0.4 % ID/g was recorded with a tumour to background ratio of 2. As the blood pool still contains high levels of activity after 5 h in mice, increased tumour uptake may occur at later time points, which might warrant further investigation.

Keywords*:* SPECT, phenylacetic acid metabolism, solid tumour, ^{131}I

Introduction

Phenylacetic acid (Fig. 1) and its physiological deprotonated form, phenylacetate, is an aromatic molecule and is present in low concentrations in human serum, as a product of phenylalanine metabolism. It is conjugated with glutamine in the liver by phenylacetyl coenzyme A, to form phenylacetylglutamine, which is excreted in the urine [1]. Phenylacetate is one of the tumour metabolism compounds that are currently studied in phase II clinical trials targeting brain tumour by reducing plasma levels of glutamine. [2].***Place for Figure 1***

Phenylacetate has been reported to have a potent anti-proliferative and anti-differentiating effect in haematological malignancies and in solid tumours at non-toxic concentrations. It has been used safely to treat children with congenital errors in urea synthesis and in patients with hyperammonaemia [1, 3]. Furthermore, it has been shown to inhibit tumour growth while sparing normal tissue and to induce phenotypic reversion and differentiation of malignant cells. The antiproliferative effect of phenylacetate has been demonstrated in numerous cancer cell lines including, prostate carcinoma [4], melanoma [5], rhabdomyosarcoma [6], breast cancer [7], pancreatic adenocarcinoma [8, 9], ovarian carcinoma [9], B-chronic lymphocytic leukaemia [10] and medullablastoma [11]. Phenylacetate showed minimal toxicity in Phase I trials of patients with hormone-refractory prostate cancer and high-grade glioma, and a partial antitumourigenic response was observed at a serum concentration of (2-10) $\times 10^{-3}$ mol/dm^{-3} [12, 13].

Increasing evidence points to phenylacetate having multiple effects on gene expression and on regulatory proteins responsible for its antineoplastic effects [14]. In fact, phenylacetate has been shown to down-regulate Bcl-2 and up-regulate bax/p21 apoptosis-related genes [9,15]. Phenylacetate also activates the human peroxisome proliferator-activated receptors (PPAR), which belong to the superfamily of nuclear steroid receptors, including retinoids, vitamin D, and thyroid hormone receptors - all important regulators of cell growth and differentiation [16, 17]. In all these studies phenylacetate exerts its effects at clinically acceptable serum concentrations ((2.5 – 10) $\times 10^{-3}$ mol/dm^{-3}) [14].

In recent years, the quest for an agent that can be used for both the diagnosis and therapy of a disease is progressing world-wide. Most notable is the exploitation of the synergism between the chemistry of the isotopes of technetium (^{99m}Tc) and rhenium (^{186}Re and ^{188}Re), in which ^{99m}Tc is used for diagnosis and ^{186}Re and ^{188}Re for therapy of the disease [18]. ^{131}I labelled compounds also fulfil the criteria of both diagnostic and therapeutic agent. Although the β-energy of ^{131}I is not ideal ($t_{½}$ = 8.02 d; γ-energy = 364 keV; ∃-energy = 0.6 - 0.8 MeV [19]), it is adequate for use in diagnostic studies using a high-energy collimator. The ∃-energy is sufficient to deliver an internal therapeutic radiation dose to the target organ or carcinoma. A further advantage of ^{131}I is that the biodistribution of the labelled phenylacetic acid and radiation exposure of a therapeutic dose to the target organ or carcinoma, can be evaluated by imaging the emitted γ-radiation. These diagnostic and therapeutic effects can be achieved using extremely small amounts of the agent, as the effects observed are determined by the radioactivity of the compound and not by the mass of material used. Radiolabelling with iodine also affords the options of ^{123}I SPECT imaging (with $t_{½}$ = 13.3 h;

γ-energy = 159 keV), ^{124}I PET imaging (with $t_{½}$ = 4.2 d; β^+-energy = 1.5 MeV) and therapeutic ^{125}I ($t_{½}$ = 59.4 d) Auger electrons.

To our knowledge, the biodistribution of ^{131}I labelled phenylacetic acid has never been evaluated in animals or humans. Due to the closeness of its structure compared to phenylacetic acid, as well as its reported biological activity, examination of the biodistribution of a ^{131}I labelled agent in animals (especially with regards to its uptake in neoplastic vs. normal tissue) was warranted. The results presented here describe the biodistribution of [^{131}I] 4-iodophenylacetic acid in healthy rats and athymic nude balb/c mice xenografted with WHCO1 cells demonstrating its potential as a diagnostic imaging and/or therapeutic agent for the treatment of neoplastic conditions.

Materials and methods

Radiosynthesis of 4-[^{131}I] iodophenylacetic acid

The radiosynthesis has been described in detail by Szucs et. al. [20]. Briefly, 0.3 mg (1 μmol) cold 4-iodophenylacetic acid was dissolved in 10 μl ethanol in a 1 ml vial. Freshly prepared ascorbic acid (0.5 mg, 3 μmol) in 20 μl water, 0.6 μmol of $CuCO_3$ catalyst and 2.1 mg (11 μmol) citric acid was dissolved in 20 μl of water and transferred to the 1 ml vial. The reaction mixture was diluted with 50 μl water and 40 μl ethanol. The reaction contents were purged with argon gas, a micro-magnetic stirrer bar was introduced and 100 μl (> 150 MBq) of the radioactive ^{131}I was added. The reaction vial was hermetically closed and the isotope exchange reaction was carried out at 150°C for one hour with vigorous stirring. After cooling, the reaction contents were transferred to the AG-MP-1M column for removal of unreacted radioiodine. The eluates (ethanol) were collected and gently evaporated under argon gas flow down to 1 ml. The residue was diluted by saline to 3 ml and filtered through a 0.22 μm Millex GV (Millipore) sterile filter into a sterile, pyrogen-free bottle with a silicon based septum. Quality control was carried out at room temperature by a Varian HPLC equipped with the Agilent Eclipse XDB-C18 column. Separation was carried out by a gradient method, using ethanol and 0.01 M ortho-phosphoric acid in water, as solvents. The flow rate was 1.0 ml/min. Twenty microlitres of the final solution was injected. The radioactivity of the sample was detected by a 30 μl flow-cell and the chemical composition by the UV-detector at 210 nm. All chemicals and solvents used were of AR or high performance liquid chromatography (HPLC) grade purity. 4-iodophenylacetic acid was purchased from Alfa Aesar L13345 with a certified purity of 97%. The ^{131}I solution was supplied by NTP Radioisotopes Pty. Ltd, Pretoria, South Africa, in a 0.05 M sodium hydroxide solution without thiosulphate or buffer solution.

Biodistribution of 4-[^{131}I]-iodophenylacetic acid in rats

All studies were performed with the approval of the Ethics Committee of the University of Pretoria in accordance with the guidelines of the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs

and Related Substances in South Africa. Four adult (12 weeks) male Sprague Dawley rats obtained from the UBPRC were each injected with 10 MBq of 4-[^{131}I]-iodophenylacetic acid. The chemical dose was 30 µg, which is a non-toxic dosage of the radiolabelled compound. The rat used for the dynamic study was anaesthetised with a subcutaneous injection of 70 mg/kg BW Ketamine hydrochloride 100 mg/ml (Anaket-V, Centaur Labs, Isando, RSA) and 0.4 mg/kg BW medetomidine hydrochloride 1 mg/ml (Domitor®, Pfizer, manufactured by Orion Pharma, Espoo, Finland). The other rats were anaesthetised with Isofluorane (Isofor®, Safe Line Pharmaceuticals (Pty) Ltd, Florida, RSA), first in an induction chamber, then maintained with a nosecone. The injection of the 4-[^{131}I]-iodophenylacetic acid was administered through a Delta Vent 26G intravenous catheter (Delta Med s.r.l., 46019 Viadona (Mn), Italy), placed in the distal third of the tail. The rats were placed in ventral recumbency on the camera and, on countdown, 4-[^{131}I]-iodophenylacetic acid was injected. Imaging was performed using an integrated SPECT-CT (Infinia-Hawkeye, GE Healthcare) in planar mode, equipped with a high-energy, parallel-hole collimator (photopeak window set at 20 %, 364 keV). A one-hour dynamic study was done on one animal, followed by static studies on the hour up to 5 hours on all four animals. Two minute static studies were performed at every hour after injection of the radiopharmaceutical, using Isofluorane to immobilise the animals. Regions of interest were drawn over the heart, lungs, liver, kidneys and a background area to determine the uptake in each organ. These values were used to generate time-activity curves to determine the biodistribution of the [^{131}I]-iodophenylacetic acid. The animals were euthanised with an overdose of Isofluorane. The brain, heart, lungs, liver, stomach, bladder, thyroid, kidneys and a portion of muscle from the left leg were removed. A 1 ml sample of blood and all urine from each were also collected. The organs and a portion thereof were weighed and the tissue radioactivity measured with an automated γ counter (NaI scintillation detector with Genie-2000 software package from Canberra Co). The remains of carcases were also counted to balance the activity in the organs with the injected dose. The percent-injected dose per gram of tissue (% ID/g) was calculated by comparison with samples of a standard dilution of the initial dose. The activity excreted was indirectly determined by subtracting the activity accounted for in all the organs from the total injected activity. All measurements were corrected for decay.

Biodistribution of 4-[^{131}I]-iodophenylacetic acid in nude balb/c xenograft mice

All studies were performed with the approval of the Animal Use and Care Committee of the University of Pretoria in accordance with the South African National Standard for the care and use of animals for scientific purposes (SANS 10386:2008). Twelve (7-8 week old) female, tumour-burdened, athymic nude balb/c mice were obtained from the University of Cape Town breeding house. Tumour inoculation was achieved by injecting 2.5×10^6 WHCO1 cells suspended in 100 ul PBS subcutaneously into the right flank of each mouse. After two weeks, the tumours were between 5 and 8 mm in size. The mice (20.2 ± 1.78 g) were each injected with 2.8 ± 0.86 MBq of 4-[^{131}I]-iodophenylacetic acid. The chemical dose was ~ 3.5 µg, non-toxic dosage of the radiolabelled compound. The 4-[^{131}I]-iodophenylacetic acid was administered directly into the tail vein in the form of a bolus injection. The mice were anaesthetised via an induction chamber containing Isofluorane, then maintained with a nosecone for cardiocentesis before euthanasia. The animals were euthanized after 5 h with a lethal dose of Isofluorane. The

tumour, heart, lungs, liver, spleen, GI tract, bladder, trachea, kidneys, abdominal fat and part of the muscle from each left hindleg were harvested. A sample of blood and all urine from each mouse was also collected. The organs and a portion thereof were weighed, and the tissue radioactivity measured with an automated γ counter (NaI scintillation detector with Genie-2000 software package from Camberra Co). The remains of carcases were also counted to balance the activity in the organs with the injected dose. The percent-injected dose per gram of tissue (% ID/g) was calculated by comparison with samples of a standard dilution of the initial dose. The activity excreted was indirectly determined by subtracting the activity accounted for in all the organs from the total injected activity. All measurements were corrected for decay.

Results

Radiolabelling of iodophenylacetic acid

It was found that inert atmosphere and adequate temperature are the key parameters for the synthesis. Fresh preparation of the ascorbic acid solution was also essential. The HPLC QC showed that the radiochemical purity of the final product was higher than 98% (Fig. 2). As described by Szucs et al. [20], the ethyl ester of iodophenylacetic acid can also form during the synthesis, the retention time being 3.5 min longer (see Fig. 2). The peak was present and constituted approximately 1.70 % of the product solution. The activity of the product was 96 MBq. The calculated specific activity was 355 Ci/mol.

Proposed place for Fig 2

Biodistribution of [^{131}I]-iodophenylacetic acid in rats and xenograft mice

A 1 h dynamic scan was recorded for one of the rats, whereafter static scans were recorded for all four rats up to 5 h The time-activity curves are presented in Fig. 3 and the $t_{½}$ values in Table 1. After completion of the scintigraphy, the rats were euthanised, dissected and the radioactivity of the organs counted. The average of the percent-injected dose per gram of tissue (% ID/g) is given in Table 2.

Similarly, the xenograft mice were euthanised 5 h after injection, dissected, and the radioactivities of the organs were counted. The average of the percent-injected dose per gram of tissue (% ID/g) is presented in Table 3 for nine subject mice. Three subjects were considered outliers (and excluded from the study), predominantly due to a high percentage of activity remaining in the tail after injection. The tumour to background ratio was calculated by comparing the muscle on the left hind leg as opposed to the right flank where the tumour was induced.

Proposed place for Fig 3 and Table 1, 2 and 3

Discussion

The labelling via isotopic exchange yielded a low specific activity of the tracer, which meant that a substantial amount of 4-iodophenylacetic acid was injected into the subject animals. This amounts to 30 µg per rat, or 75 µg/kg, or 1.1 µg/ml blood (mass of rats ~400 g and assuming 7 % of body weight is blood). The LD_{50} value for phenylacetic acid is 1600 mg/kg for intraperitoneal injection in rats [21], which is orders of magnitude higher than the amount administered in these experiments. Likewise the level of phenylacetic acid in normal tissue is 16.8 µg/ml [22], which is also an order of magnitude higher than the amount of 4-iodophenylacetic acid injected. This implies that the biodistribution of the tracer may not have been influenced by its metabolic product due to equilibrium with endogenous phenylacetic acid in the body.

It is known from the work with omega-iodophenyl substituted fatty acids that these derivatives are metabolised to iodobenzoic acid or iodophenylacetic acid, which is rapidly excreted through the kidneys [23]. Therefore, one would expect that, in healthy rats, 4-[^{131}I]-iodophenylacetic acid would be excreted and not accumulate in a target organ. Indeed, this is what we have demonstrated in this study, although the excretion observed was not as rapid as that of other phosphonate-based radiopharmaceuticals reported previously [24].

The long half-life for excretion from the kidneys seems contradictory to this, but the values in the kidney are not high at the start and do not increase over time either, yet there is clearance from the blood pool and liver (Fig. 3). After 5 h, 55 % of the activity was excreted, most likely via the kidneys, as the stomach shows very low % ID/g. This is supported by the literature [25] that indicates that iodophenylacetic acid is conjugated to amino acids that facilitate its excretion into the urine, similar to that of benzoic acid, which conjugates with glycine.

The standard deviations for the % ID/g (Table 2) between the four rats are acceptable, except in the case of the bladder. This may be explained by urine left behind in the bladder (although procedures were followed to remove all urine after euthanasia) and the very low mass of the bladder (avg. mass was 126 ± 8 mg) making errors unavoidable. The same argument applies to the thyroid (although the standard deviation is only 20 %), which also has a small mass (avg. mass was 194 ± 23 mg). The % ID/g for the thyroid is 4.19 %, which is high, but compares well with the 8.53 % (at 180 min) achieved by Mokler et al. [25] for the ^{125}I labelled phenylfatty acid β-methyl-para-iodophenylpentadecanoic acid (BMIPP). In terms of the overall % ID, the value for the thyroid is only 0.86 %. HPLC analyses of 4-[^{131}I]-iodophenylacetic acid were performed up to 5 d later and showed no free iodine formation over this period (data not shown). However, it cannot be ruled out that enzymatic degradation of 4-[^{131}I]-iodophenylacetic acid may be responsible for the uptake in the thyroid, although no evidence for the release of iodide by the metabolism of 4-iodophenylacetic acid has been reported in the literature. The bladder and thyroid, therefore, seem not to be target organs of 4-[^{131}I]-iodophenylacetic acid. [^{125}I]-BMIPP was evaluated as a myocardial imaging agent [25] and metabolism thereof by the myocardium was demonstrated. The initial high value for the heart (including cardiac blood pool) in this study (Fig. 3) might be indicative of the same mechanism for 4-[^{131}I]-

iodophenylacetic acid, but - after 5 h - the remainder in the heart was low (Table 3), which agrees with findings of the [^{131}I]-BMIPP study [25].

Based on the observation that no particular target organ was found for the 4-[^{131}I]-iodophenylacetic acid in this study, a study with tumour-containing animals was pursued. From Table 3, it is clear that - for the xenograft mice - all organs contain higher % ID/g after 5 h than for the rats. It seems that localisation was not complete, especially if one considers the high blood values and that only 32% of the injected dose had been excreted. The standard deviations for the % ID/g (Table 3) between the nine mice are low (lower than for the rats), except in the case of the bladder and lung. The results for the lungs are probably an artefact due to the high blood levels of 4-[^{131}I]-iodophenylacetic acid, which influence the total counts of lung tissue. The reason for the bladder results is the same as for the rats as well as for the high thyroid uptake. As the organ is so small, a part of the trachea was taken as well. In terms of the overall % ID, the value for this organ part is only 0.2%. The tumour uptake was 4 ± 0.4 % and the tumour to background ratio (comparing tumour uptake vs. the muscle tissue on the opposite flank) was 2. This was not as high as expected, but is mostly likely due to the slower localisation/excretion of radiolabelled agent in the mice than in the rats. This difference in biodistribution might be due to different renal function or metabolism of the two species. Indeed, the investigation should also be carried out in wildtype nude mice to allow effective comparison. As the blood still contains high levels of activity (which is assumed to be 4-[^{131}I]-iodophenylacetic acid) after 5 h in mice, increased tumour uptake may be expected later on which might warrant further investigation.

Conclusions

4-[^{131}I]-iodophenylacetic acid was prepared successfully and the biodistribution in rats recorded. As expected, no target organ was found after 5 h (although at the early stages a high cardiac blood pool uptake was recorded) with fast excretion from all organs via the kidney into the urine. In the xenograft mice study, a 4 % tumour uptake and a tumour to background ratio of 2 was recorded after 5 h, although high activity levels in the blood still remained at that time which might warrant further investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table **1:**Half life of 4-[^{131}I]-iodophenylacetic acid in various body compartments of the rats.

Organ	**Cardiac blood pool**	**Liver**	**Lung**	**Kidney**	**Background**
$\mathbf{t_{½}}$ (min)	182	204	117	> 300	> 300

*values calculated by fitting an exponential curve to the data points (as presented in Fig. 3) for the time period 10-60 min

Table **2**: The biodistribution of 4-[^{131}I]-iodophenylacetic acid in rats as expressed in %ID/g, 5 h after injection (n=4).

Organ	**Average %ID/g**	**SD (%ID/g)**	**%SD**
blood	1.53	0.28	19
heart	0.67	0.10	14
spleen	0.33	0.05	17
lung	1.08	0.18	17
liver	0.63	0.14	22
R kidney	1.05	0.24	23
L kidney	1.05	0.24	23
thyroid	4.19	0.84	20
stomach	0.34	0.08	24
muscle	0.17	0.04	23
bladder	2.28	1.35	60
excretion*	55	8	-

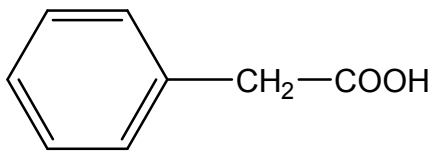
* % excretion has been indirectly calculated by subtracting the activity accounted for in all the organs from the total activity injected.

Table **3.** The biodistribution of 4-[^{131}I]-iodophenylacetic acid in xenograft mice as expressed in %ID/g, 5 h after injection (n = 9).

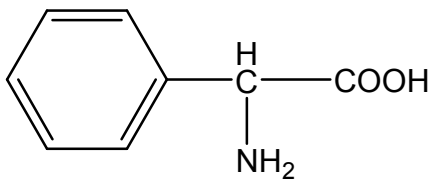
Organ	**Average %ID/g**	**SD (%ID/g)**	**%SD**
blood	17.6	2.0	11
heart	6.18	0.77	12
spleen	1.73	0.19	11
lung	6.27	2.28	36
liver	3.58	0.49	13
kidneys	5.09	0.58	11
fat	2.30	0.53	23
trachea	9.73	1.29	13
GI tract	1.94	0.32	16
bladder	13.1	7.5	57
muscle	2.04	0.18	9
tumour	4.10	0.43	10
T:B[#]	2.0	-	-
excretion*	32	10	-

***** % excretion has been indirectly calculated by subtracting the activity accounted for in all the organs from the total injected activity.

[#] Tumour to background ratio when comparing tumour uptake vs. the same muscle tissue on the opposite flank



Phenylacetic acid



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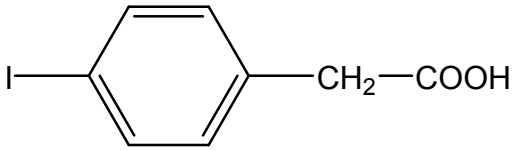
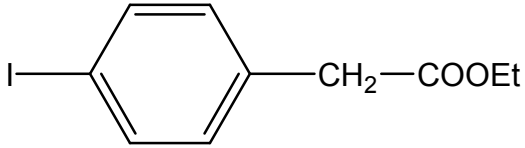


4-iodophenylacetic acid



Ethyl 2-(4-iodophenyl)acetate

Figure 1. The structures of 4-iodophenylacetic4-Iodophenylacetic acid, phenylacetic acid and phenylalanine.

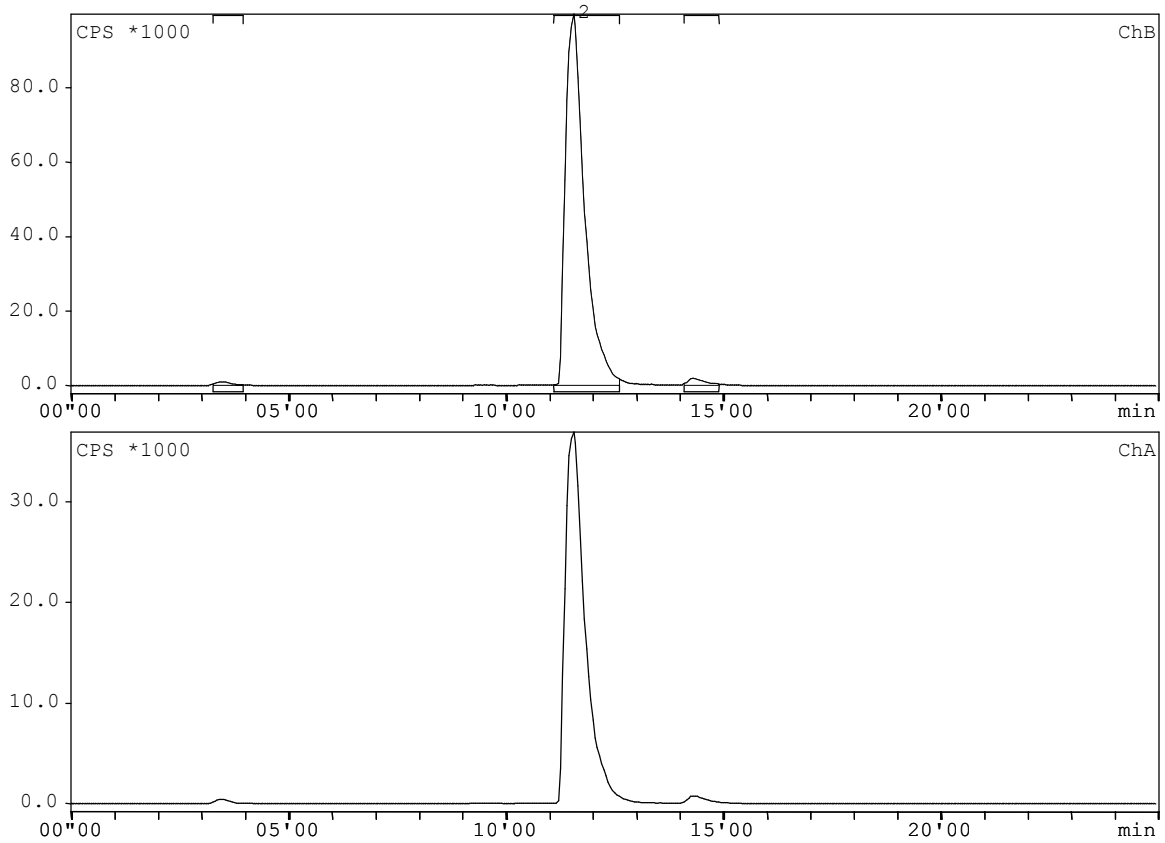


Figure 2. Radiochromatogram (top) of 4-[^{131}I]-iodophenylacetic acid and UV chromatogram (bottom) of the cold iodophenylacetic acid standard

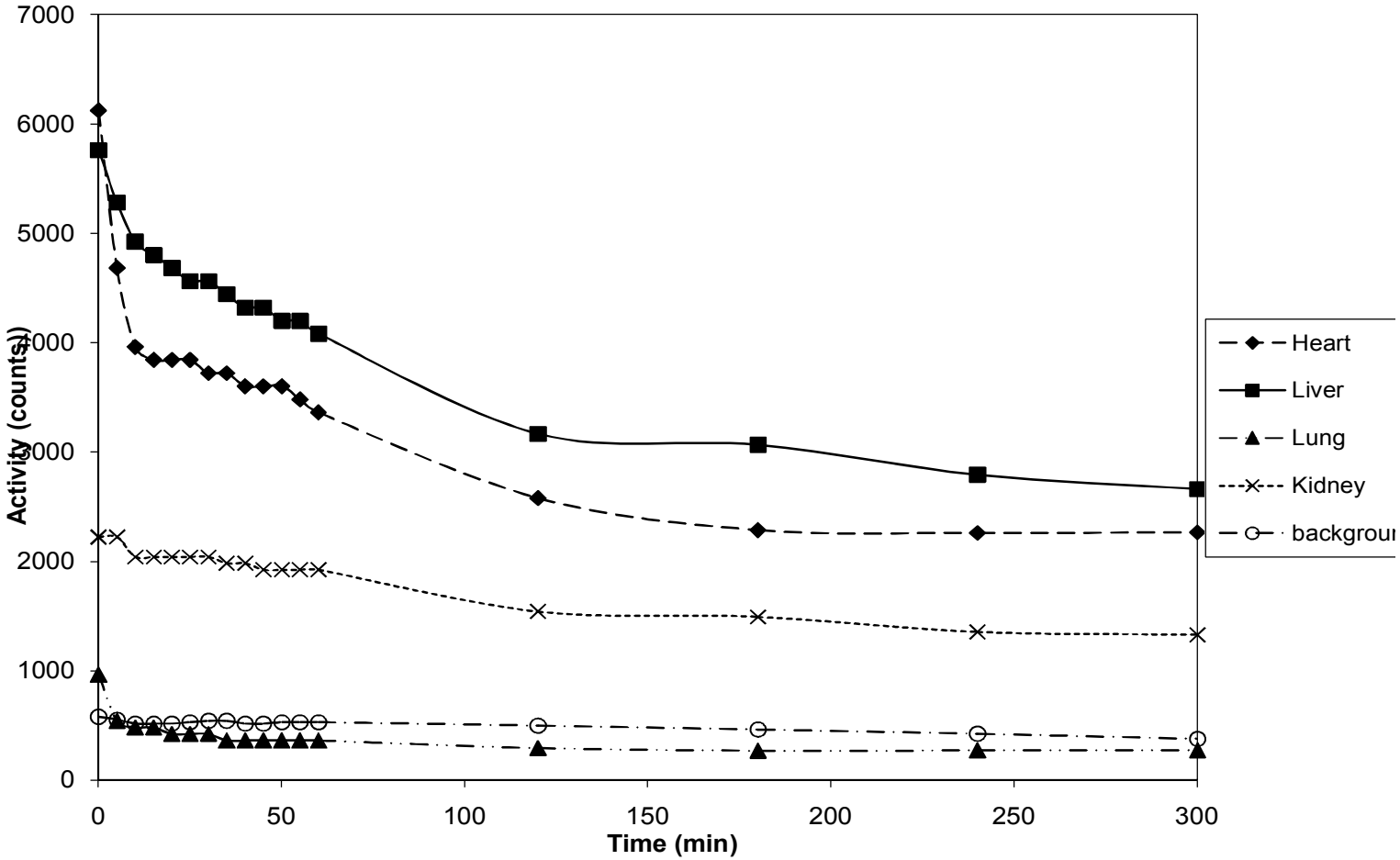


Figure 3. Time activity curves (n=1 for 0-55 min and n=4 for 60-300 min) for cardiac blood pool (-◆-), liver (-■-), lung (-▲-), kidney (-✕-) and background (-●-) as counts vs. time in minutes.