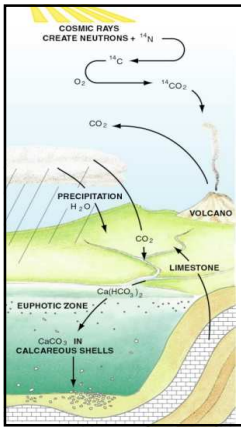
Europejski program badań farmakokinetycznych (w populacji pediatrycznej) **z zastosowaniem AMS - udział Instytutu Farmaceutycznego**

Leki stosowane w pediatrii
– problemy w opracowaniu, badaniach klinicznych, dopuszczaniu do obrotu, wytwarzaniu i stosowaniu
Konferencja Naukowo-Szkoleniowa, CZD, 06-07 marca 2013



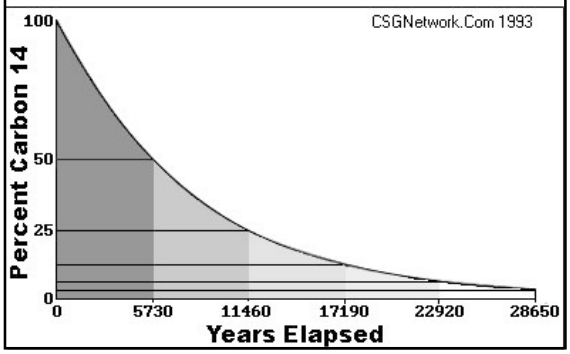
$^{14}N + 1n \rightarrow {}^{14}C + {}^{1}H$

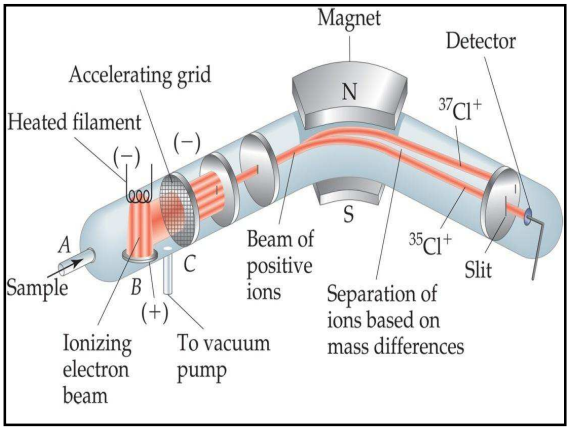
$^{14}C \rightarrow {}^{14}N + e^-$

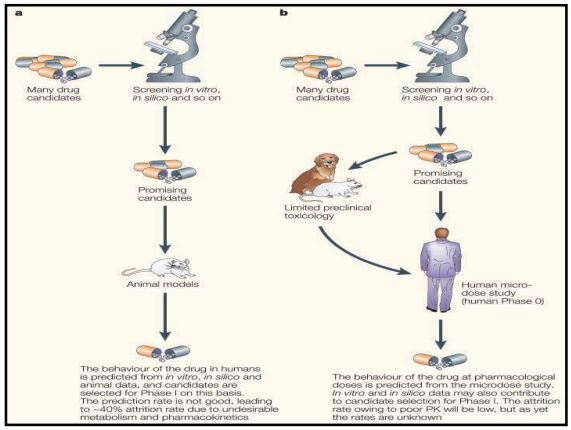
^{14}C detection: beta counting *versus* atom counting

- Isotopic abundance of ^{12}C 98.9 %
- Isotopic abundance of ^{13}C 1.1 %
- Isotopic abundance of ^{14}C in modern (pre-bomb) carbon: 1.2×10^{-12} $^{14}C/^{12}C$
- ^{14}C content of 1 mg of modern carbon 6×10^{7} ^{14}C atoms
- Half-life of ^{14}C = 5730 ± 40 y (low energy beta emission)
- Activity of 1 mg of modern carbon 0.8 ^{14}C decays/h
- Activity of 1 mg of 50,000-year old carbon 17 ^{14}C decays/y
- Negative ion $^{12}C^-$ output from 1 mg of modern carbon 1.9×10^{14} $^{12}C^-$ ions/s
- Negative ion $^{14}C^-$ output from 1 mg of modern carbon 230 $^{14}C^-$ ions/s
- Counting rate of ^{14}C in the final AMS detector : 115 $^{14}C3^+$ ions/s (4.1×10^{5} $^{14}C3^+$ ions/h)
- Counting rate of ^{14}C from 1 mg of 50,000-year old carbon 970 $^{14}C3^+$ ions/h
- Gain in ^{14}C detection sensitivity with AMS 5.1×10^{5} AMS/ß⁻counting.

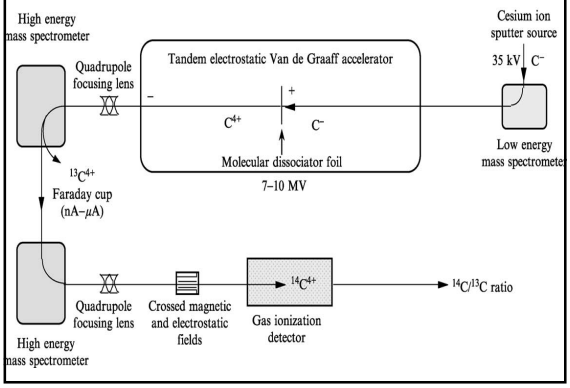
Principle of carbon radiodating

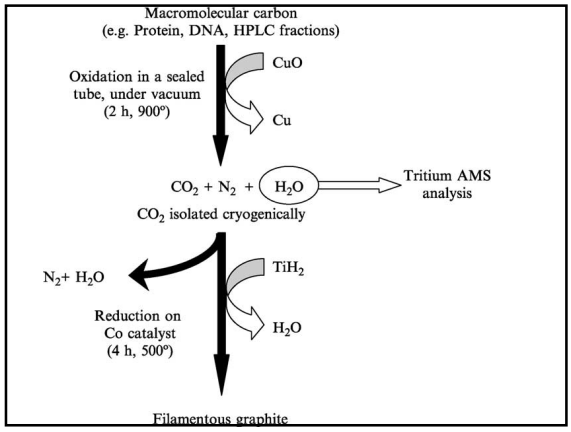


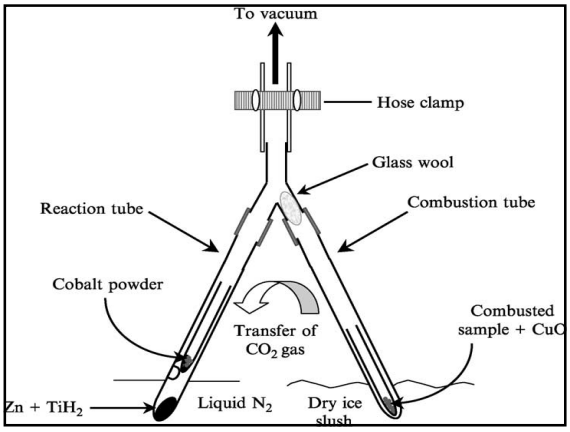




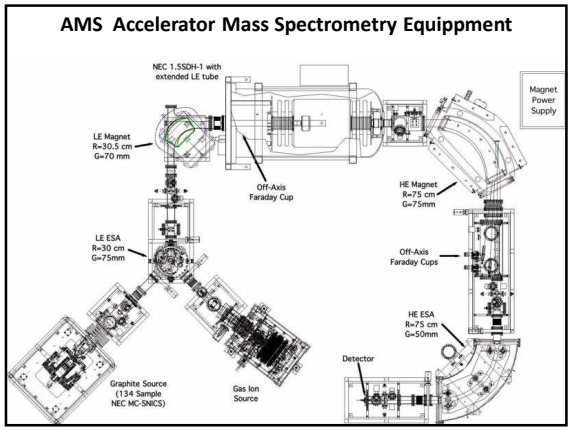
AMS Accelerator Mass Spectrometry Principle







AMS Accelerator Mass Spectrometry Equippment



AMS Facility at Xceleron Ltd., York, UK

As the half-life of 14C is 5730 years, only 0.012% of 14C atoms decay in a given year. To observe one disintegration per second, 4.3 x 10^9 14C atoms must be present.
For precise AMS count approx. 1000 14C atoms need to be recorded! (atto – zepto range)

Accelerator Mass Spectrometry Accuracy

AMS (Accelerator MassSpectrometry) is an extremely sensitive analytical technique with the ability to detect drugs down to 10^{-18} (attogram) to 10^{-21}(zeptogram) moles AMS has the potential for analysing drug and metabolite concentrations in body fluids withdrawn at time intervals after dosing. AMS requires isotopically labeled candidate drugs, in contrast to liquid chromatography/mass spectrometry (LC/MS). However, the latter method does not currently have the necessary sensitivity to accurately measure drug concentrations at microdoses. Besides, the amount of radioactivity used is so low (nCi/Bqs) that it is not considered to be radioactive dose from a regulatory perspective. AMS measures isotopes at the single atom level and allows the determination of isotope ratio 12C/14C with **exquisite precision.** It measures isotope atoms by mass and not decay. As a result, AMS sensitivity is 1 million times higher than liquid scintillation counting and up to hundred thousand times higher than conventional mass spectrometry.

European Union Microdose AMS Partnership Programme (EUMAPP 2006 - 2008) – Background

The microdosing approach offers the opportunity of early human screening of many more drug candidates offering greater predictability versus animal and/or *in vitro* models. Hence, human microdosing studies offer the promise of (1) improved candidate selection (2) reduced attrition rates (3) safer clinical studies and (4) a potential reduction in the use of animals in early clinical development. The microdosing approach could by this means potentially reduce the time of pre-clinical testing from 18 months to 4 to 6 months and cut down the associated expenses by 10 times.

European Union Microdose AMS Partnership Programme (EUMAPP; 2006 - 2008) experience:

1) **Acetaminofen (Paracetamol)**
2) **Clarithromycin**
3) **Fexofenadine (-)**
4) **Phenobarbital**
5) **Propafenone**
6) **Sumatriptan**
7) **S-19812-1 (drug candidate) [IMPD documentation completed]**
8) **S-23361-1 (drug candidate active metabolite)**

Microdosing (< 100 microgram, single dose) validated as method for collecting PK parameters and information about metabolism; accepted as Phase 0 Clinical Study

The EUMAPP Consortium

1 XCELERON , Colin GARNER; York, UK
2 Institut de Recherches Internationales (IRIS), Roeline JOCHEMSEN; Courbevoie, FR
3 Pharma Bio-Research Group B.V. (PBR), Berend OOSTERHUIS ; Zuidlaren, NL
4 University of Manchester (CAPKR), Brian HOUSTON; Manchester , UK
5 Pharmaceutical Research Institute (PRI), Grzegorz GRYNKIEWICZ; Warsaw, PL
6 CYPROTEX Discovery Ltd (CYPROTEX) Simon THOMAS; Macclesfield, UK
7 University of Lund (L.U.), Kristina STENSTRÖM; Lund, SE
8 European Federation for Pharmaceutical Sciences (EUFEPS) O.J. BJERRUM; Stockholm, SE
9 Foundation for the Evaluation of Ethics in Biomedical Research (BEBO), Rokus DE ZEEUW; Assen NL
10 ACIES, David KOUBI; Lyon, FR

PRIOMEDCHILD (Pamper)

Standard procedure for AMS – microdosing study on selected drug API:

1. **Collecting information on drug metabolism**
2. **Critical survey of synthetic methods and choice of labeling proceure**
3. **Securing dedicated personnel, space and equipment**
4. **Cold synthesis, elaboration of analytical methods**
5. **Scale-down to a single miligram level**
6. **Execution of synthesis with use of radiotracer**
7. **Purification of the labeled sample**
8. **Starting stability study**
9. **Certification**
10. **Distribution to the project partners; storage of archive samples**

The PAMPER Consortium
ERA-NET PRIOMEDCHILD Project 40-41800-98-022 (2011-2014)

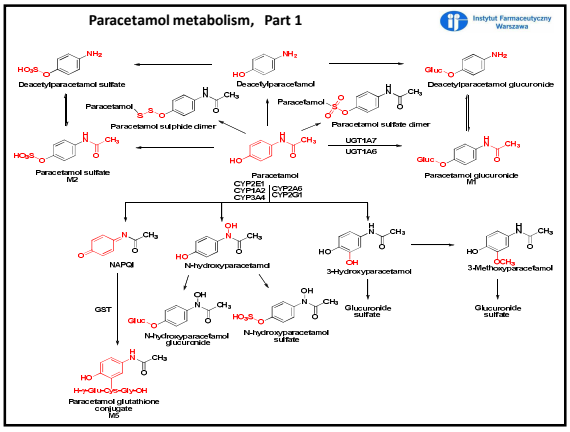
- **University of Liverpool (UoLi), Liverpool UK; Prof. Kevin Park** (project Coordinator)
- **Garner Consulting Services (GCS), York, UK; Prof. Colin Garner** (project Manager)
- **Alderhey Children's Hospital (AHCH), Liverpool, UK; Dr. T. Nunn**
- **Tartu University Hospital (TUH), Tartu, Estonia; Dr. Heili Varendi**
- **Pharmaceutical Research Institute (PRI), Warsaw, Poland; Prof. G. Grynkiewicz**
- **Addenbrooke's Hospital, Cambridge, UK; Dr. K. Solanki**
- **TNO, Delft, The Netherlands; Dr. Wouter Vaes**
- **Good Glinical Practice Alliance-Europe (GCPA), Kessel-Lo, Belgium; F. Crawley**
- **Hammersmith Medicines Research (HMR), London, UK; Dr. M. Boyce**

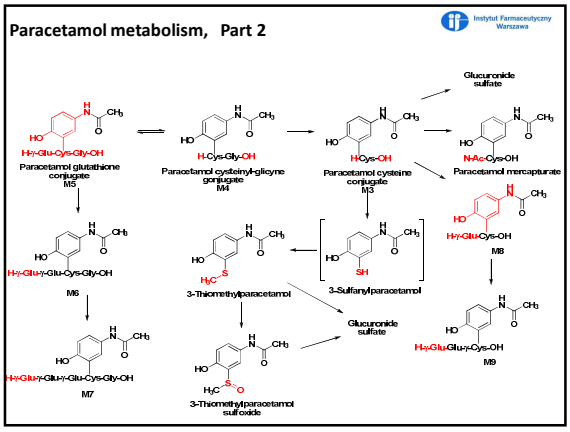
ERA-NET PRIOMEDCHILD

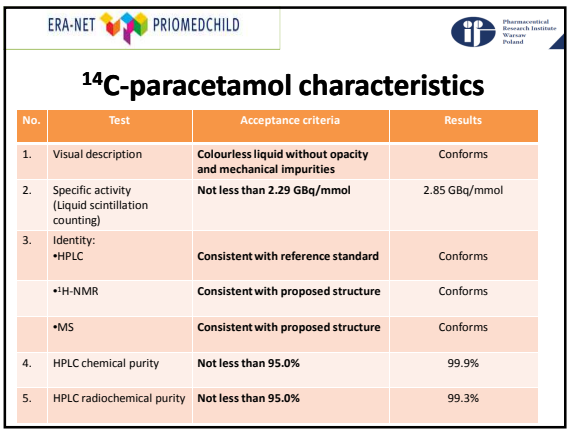
Pharmaceutical Research Institute Warsaw Poland

^{14}C-PARACETAMOL

Design of clinical studies: Therapeutic dosing; radioactive microtracing

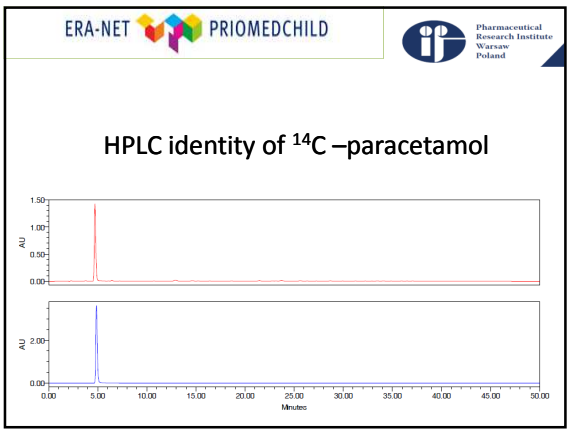




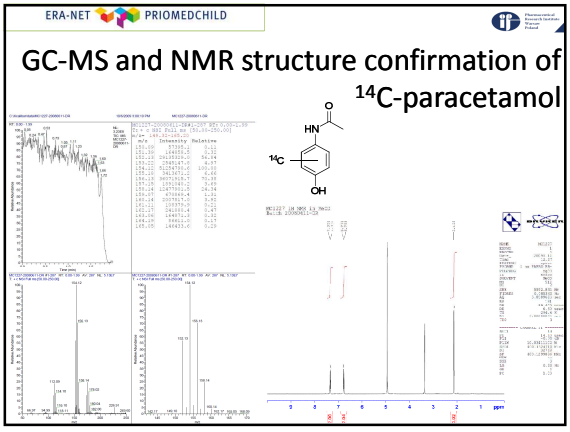


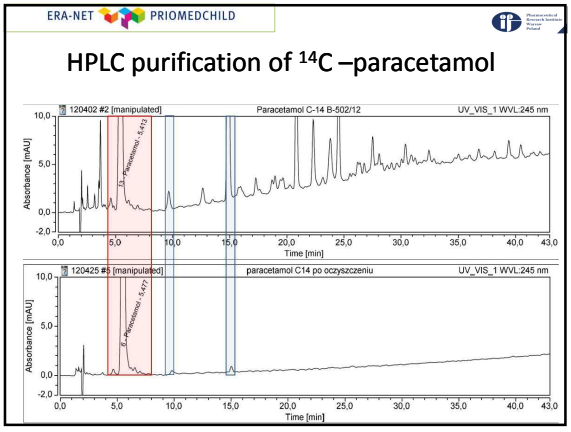
^{14}C-paracetamol characteristics

No.	Test	Acceptance criteria	Results
1.	Visual description	**Colourless liquid without opacity and mechanical impurities**	Conforms
2.	Specific activity (Liquid scintillation counting)	**Not less than 2.29 GBq/mmol**	2.85 GBq/mmol
3.	Identity: •HPLC	**Consistent with reference standard**	Conforms
	•^{1}H-NMR	**Consistent with proposed structure**	Conforms
	•MS	**Consistent with proposed structure**	Conforms
4.	HPLC chemical purity	**Not less than 95.0%**	99.9%
5.	HPLC radiochemical purity	**Not less than 95.0%**	99.3%



HPLC identity of ^{14}C –paracetamol





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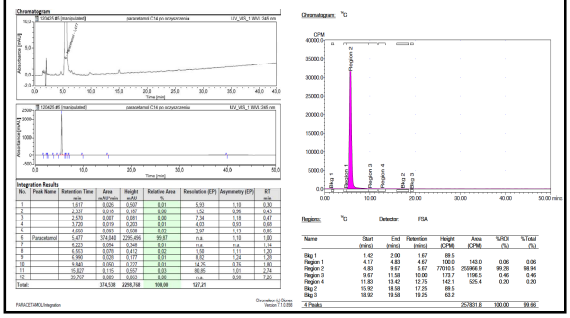
Chemical and radiochemical purity of ^{14}C – paracetamol



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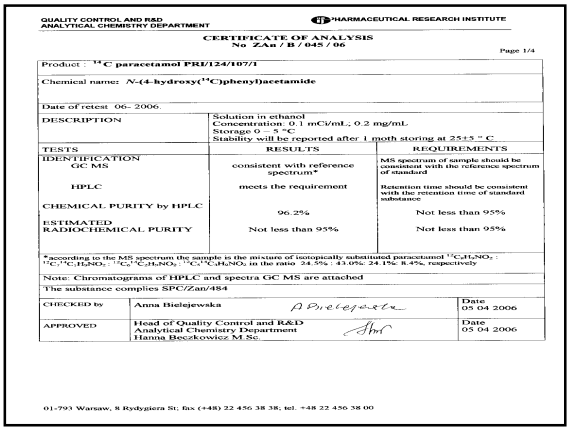
Stability study of ^{14}C –paracetamol

^{14}C-paracetamol, 2.85 GBq/mmol (77 mCi/mmol), batch No.: 153-064-077-A-20080611-DR

Storage contition: - 20 ºC — Stability initiation date: May 2012

Test	Requirements	Initial	1 month June 2012	2 months July 2012	3 months August 2012
Description	Colourless, clear liquid without opacity and mechanical impurities	Colourless, clear liquid without opacity and mechanical impurities	Colourless, clear liquid without opacity and mechanical impurities	Colourless, clear liquid without opacity and mechanical impurities	Colourless, clear liquid without opacity and mechanical impurities
Identity, HPLC	Retention time should be consistent with retention time of reference standard	Retention time is consistent with retention time of reference standard	Retention time is consistent with retention time of reference standard	Retention time is consistent with retention time of reference standard	Retention time is consistent with retention time of reference standard
Chemical purity HPLC	Not less than 95.0 %	99.9 %	97.8 %	97.0 %	95.7 %
Radiochem. purity , HPLC	Not less than 95.0 %	99.3 %	98.6 %	97.8 %	96.6 %



QUALITY CONTROL AND R&D
ANALYTICAL CHEMISTRY DEPARTMENT

PHARMACEUTICAL RESEARCH INSTITUTE

CERTIFICATE OF ANALYSIS
No ZAn / B / 045 / 06

Page 1/4

Product : ^{14}C paracetamol PRI/124/107/1

Chemical name: *N*-(4-hydroxy(^{14}C)phenyl)acetamide

Date of retest 06- 2006.

DESCRIPTION	Solution in ethanol Concentration: 0.1 mCi/mL; 0.2 mg/mL Storage 0 – 5 °C; Stability will be reported after 1 moth storing at 25±5 ° C	
TESTS	RESULTS	REQUIREMENTS
IDENTIFICATION GC MS	consistent with reference spectrum*	MS spectrum of sample should be consistent with the reference spectrum of standard
HPLC	meets the requirement	Retention time should be consistent with the retention time of standard substance
CHEMICAL PURITY by HPLC	96.2%	Not less than 95%
ESTIMATED RADIOCHEMICAL PURITY	Not less than 95%	Not less than 95%

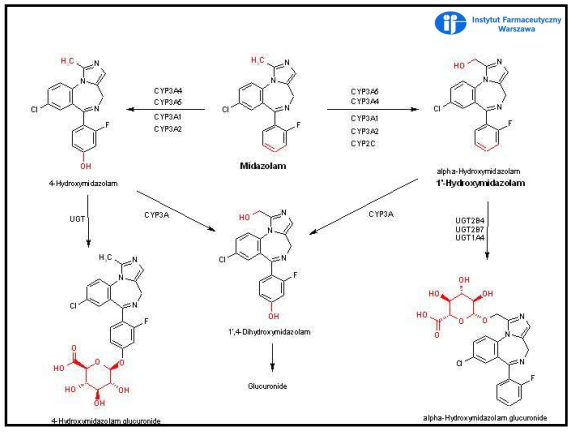
*according to the MS spectrum the sample is the mixture of isotopically substituted paracetamol $^{12}C_8H_9NO_2$: $^{14}C^{12}C_7H_9NO_2$: $^{12}C^{14}C_7H_9NO_2$ in the ratio 54.5% : 43.0% 24.1% 8.4%, respectively

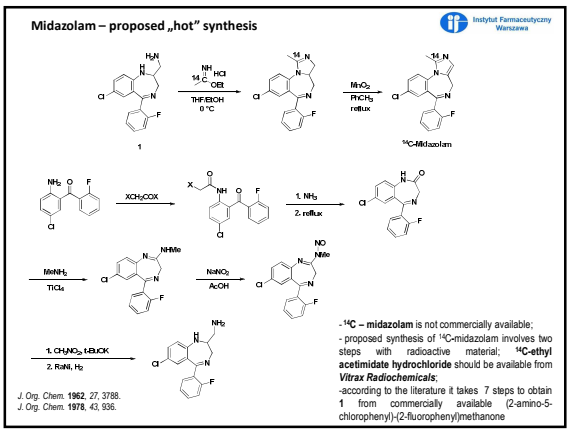
Note: Chromatograms of HPLC and spectra GC MS are attached

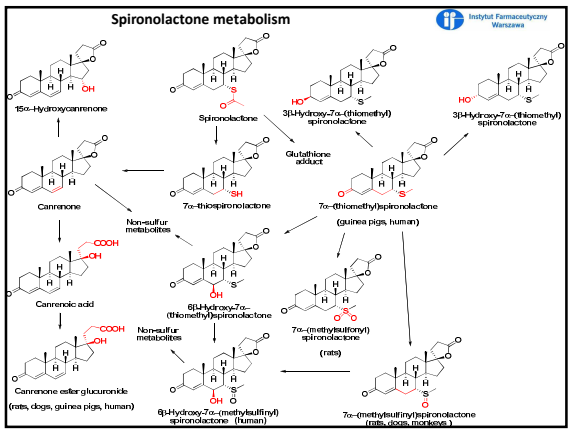
The substance complies SPC/Zan/484

CHECKED by	Anna Bielejewska	Date 05 04 2006
APPROVED	Head of Quality Control and R&D Analytical Chemistry Department Hanna Beczkowicz M.Sc.	Date 05 04 2006

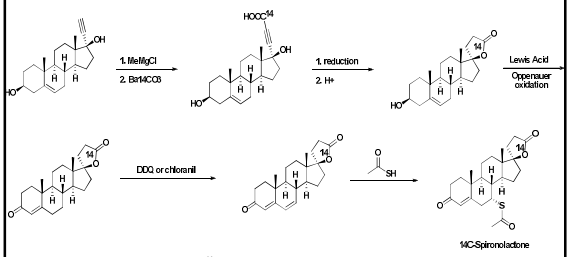
01-793 Warsaw, 8 Rydygiera St; fax (+48) 22 456 38 38; tel. +48 22 456 38 00







Spironolactone – proposed synthesis



- **^{14}C – spironolactone** is not commercially available;
- proposed synthesis of ^{14}C-spironolactone (5 steps involving radiactive materials) is known in the literature; ^{14}C has to be introduced at the begining as a **$^{14}CO_2$**
- **$Ba^{14}CO_3$** is available from ***Vitrax Radiochemicals***;
- synthesis starts from ***trans*-dehydroandrosterone** (not shown; commercially available)

J. Label Compd. Radiopharm. **1988**, *25*, 515.
J. Org. Chem. **1959**, *24*, 1109.
J. Org. Chem. **1959**, *24*, 743.
J. Am. Chem. Soc **1957**, *79*, 4808.

Bioanalysis. 2010 March; 2(3):429-40. doi: 10.4155/bio.10.6.

Practical experience of using human microdosing with AMS analysis to obtain early human drug metabolism and PK data.

Garner RC., Xceleron Ltd, The Biocentre, Innovation Way, York, YO10 5NY, UK.
garner.consulting@virgin.net

The background to human microdosing or Phase 0 studies is reviewed, focusing particularly on the information that such studies can provide in the context of exploratory clinical development. Examples are provided of the microdose-validation studies known as the Consortium for Resourcing and Evaluating AMS Microdosing trial and EU Microdosing AMS Partnership Programme, which demonstrated that there was good dose proportionality between microdose and pharmacological dose pharmacokinetics. When microdosing was applied to ten development drugs, it was found that all ten molecules showed dose proportionality between the microdose and the pharmacological dose. The majority of microdose studies have used accelerator mass spectrometry (AMS) analysis and only these studies that are considered here; AMS provides information on all metabolites, even if these are minor. There is now sufficient scientific data to justify microdose studies being routinely conducted as part of the drug-development process.

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Pharmaceutical Research Institute Warsaw Poland

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