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Short Communication

A preliminary evaluation of accelerator mass spectrometry in the biomedical field

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1. Introduction

Accelerator mass spectrometry (AMS) is an established technology, which enables the efficient measurement of very small quantities of rare and long-lived isotopes, such as ^{14}C, with high precision [1,2]. Thus, ^{14}C can be detected at the parts per 10^{15} sensitivity in milligram samples and this is orders of magnitude lower than that achievable by conventional decay counting techniques, such as liquid scintillation counting. Most instruments to date have been large and are usually sited in centres dedicated to chronometry or nuclear chemistry measurements. In recent years, AMS instruments have been applied mainly in the geochemical and archaeological areas [2,3], such as for radiocarbon dating, but more recently at the Lawrence Livermore National Laboratory (LLNL), this technology has been used for bioanalytical applications. In practice, the $^{14}C/^{13}C$ ratio is measured in the accelerator mass spectrometer, after converting the sample off-line into graphite, via CO_2. Experience to date at LLNL indicates that a level as low as that equivalent to 0.1 dpm per ml g^{-1} can be determined. We wished to evaluate the potential of this technology in the field of drug metabolism, by measuring the ^{14}C content of [^{14}C]- drug-containing samples of plasma and urine by AMS and comparing the data with previous liquid scintillation counting results from (a) a study on [^{14}C]-candoxatril in man and (b) a study on [^{14}C]-dofetilide in the rat. Candoxatril and dofetilide are Pfizer compounds in drug development.

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2. Experimental

2.1. [14C]-Candoxatril in human samples

In a study carried out 6 months earlier, in which human volunteers received a single oral dose of 200 mg candoxatril labelled with 50 μCi of ^{14}C, samples of plasma and urine were collected at specified times and radioactive content was measured in duplicate samples by liquid scintillation counting (Emulsifier-Safe scintillator in a Wallac 1409 counter). Since that time, the samples were stored at ~ -20°C, which is known not to affect ^{14}C content. For the purposes of the current study, samples of plasma from one subject were diluted with control plasma, 500-, 5000- and 50 000-fold for AMS analysis at LLNL. Samples of urine from the same volunteer were diluted 50 000-fold with control urine, also prior to AMS analysis. These dilutions were chosen to determine whether ^{14}C content could be measured in samples which, effectively, were from subjects given nanocurie doses. Single aliquots (25 μl) of the diluted samples were processed to graphite, using a two-step method that minimises the potential for sample to sample contamination [4]. In brief, samples are oxidised to CO_2 in the presence of copper oxide at 900°C and the resultant CO_2 was then reduced to filamentous graphite on cobalt, in the presence of zinc powder and titanium hydride at 500–550°C. Radiocarbon was then measured in the graphite using previously published protocols [5,6].

2.2. [14C]-Dofetilide in rats

In a previous study, two rats (Charles River; ~ 200 g) had received a single oral dose of dofetilide (5 mg kg^{-1}) in aqueous solution; the drug was labelled with 8 μCi of [^{14}C]-drug. The animals were housed in metabolism cages and urine samples were collected separately for analysis by conventional liquid scintillation counting. Duplicate samples were measured (Ecoscint A scintillator in a Wallac 1409). In the current and analogous study, two rats received the same oral dose of dofetilide, which in this instance, was labelled with 26 pCi (57 dpm) of [^{14}C]-drug. This amount was chosen to determine whether ^{14}C content could be measured in urines from animals given very small quantities of radioactivity. In this case, single aliquots of the urines were analysed by AMS at the Livermore laboratory, as in Section 2.1.

3. Results

The data on the urinary excretion of radioactivity in a subject given oral [^{14}C]-candoxatril are shown in Table 1. The amounts excreted were calculated as the measured count rate per ml sample multiplied by the volume of urine excreted in the particular time period and divided by the effective radioactive dose. There is close agreement between the two sets of data obtained by, (a) liquid scintillation counting of actual samples, and (b) AMS analysis of 50 000-fold diluted samples. In the latter case, the diluted sample was equivalent to the volunteer receiving only 1 nCi of [^{14}C]-compound and radioactivity levels were equivalent to 0.5–0.6 dpm ml^{-1}.

Plasma concentrations were not measurable by AMS following 50 000-fold dilution, since count rates were indistinguishable from background, but were measured after 5000- and 500-fold dilution. There is reasonable agreement (Table 2) between the values expected from dividing the value obtained by liquid scintillation counting by the dilution factor and that measured by AMS. The sample contamination referred to, resulted from a small spillage of radioactive material in the close vicinity of the diluted samples and this highlights the need for 'clean-bench' techniques when using this highly sensitive methodology. The urinary excretion of radioactivity in the rats given [^{14}C]-dofetilide is shown in Table 3 and, again, there is

Table 1
Urinary excretion (% dose) of radioactivity in a subject given [^{14}C]-candoxatril

Collection period (h)	50 μCi (LSC)	50 000-fold dilution (AMS)
0–4	28.6	31.1
4–8	4.6	4.5
8–12	<1	ND

Table 2
AMS analysis of plasma radioactivity (μg equivalents candoxatril ml^{-1}) after dilution of conventional samples

Sample time (h)	Dilution			
	500-fold		5000-fold	
	Expected	Found	Expected	Found
1	2.78	2.36	0.28	0.22
2	1.73	1.53	0.17	0.14
3	0.83	0.88	0.08	0.18[b]
4	0.42	—[a]	0.04	5.21[b]

[a] Sample tube broken.
[b] Contamination.

close agreement in the results from the two experiments. This indicates that it is feasible to use doses of radioactive compounds as low as 26 pCi of [^{14}C] per animal and still obtain useful excretion data. In this case the levels determined by AMS were equivalent to 0.13–0.2 dpm ml^{-1} in the 24–48 h samples.

In conclusion, the results of these preliminary studies indicate that the use of AMS has great potential in the fields of drug metabolism and related areas. In particular, it will enable the administration to humans of very low quantities of radioactivity, e.g. low nCi doses of ^{14}C. Thus, in general, this will lead to body exposures of less than 1 microSievert, which is so low that in the UK, authorisation for such a study by the Department of Health (via the Administration of Radioactive Substances Advisory Committee) is not required. In addition, the disposal of these very small quantities of radioisotopes is much less of an environmental issue than that associated with the μCi quantities which are currently used in human drug metabolism studies.

Table 3
Urinary excretion (% dose) of radioactivity in groups of rats given [^{14}C]-dofetilide

Collection period (h)	8 μCi (LSC)		26 pCi (AMS)	
	Rat 1	Rat 2	Rat 3	Rat 4
0–24	39.4	46.0	36.3	38.2
24–48	3.3	3.3	3.3	2.9

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