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Introduction

In recent years there has been increasing interest in evaluating the safety of transgenic plants regarding 'unintended effects' of genetic engineering. Such modifications of the chemical composition may affect the safety or nutritional status of these crops. ¹H-NMR and GC-MS have been investigated as techniques for the profiling of metabolites. Data analysis using multivariate methods will assist in establishing whether these changes are significant.

Profiling by NMR

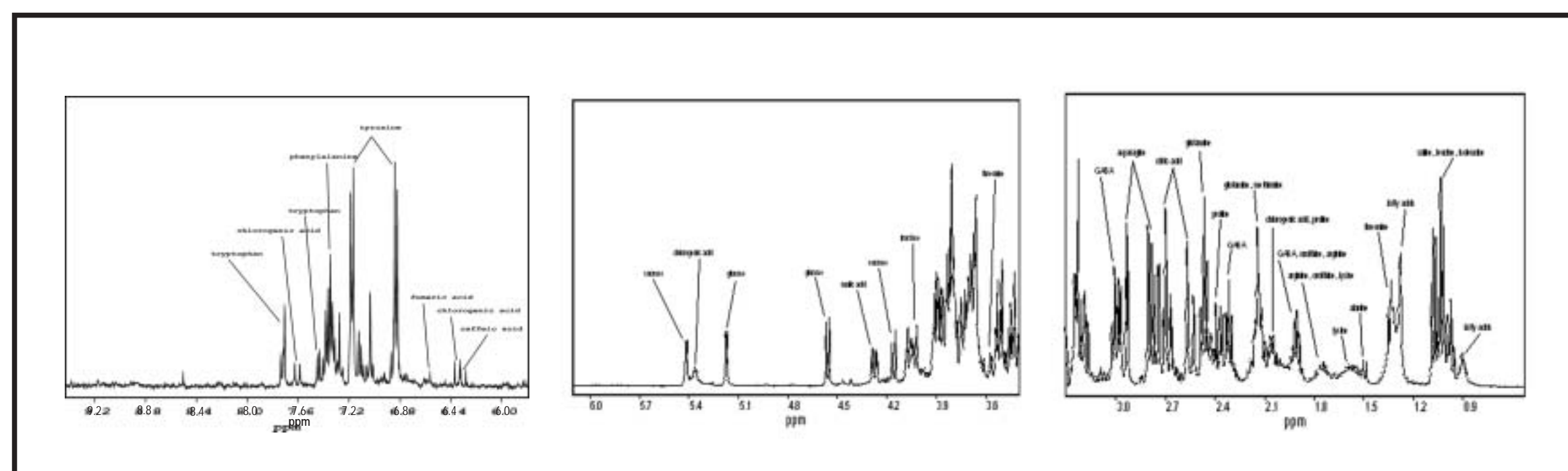


Figure 1 An example ¹H-NMR spectrum of an extracted potato sample showing peak assignments.

Whole freeze-dried potato samples were extracted with 70% deuterated methanol/water containing 100 mM phosphate buffer at room temperature for 30 minutes. The resulting extracts were analysed by NMR (at 400MHz). An example of the data obtained and some assignments (identified from analysis of reference spectra and 2D-NMR) are shown in Figure 1.

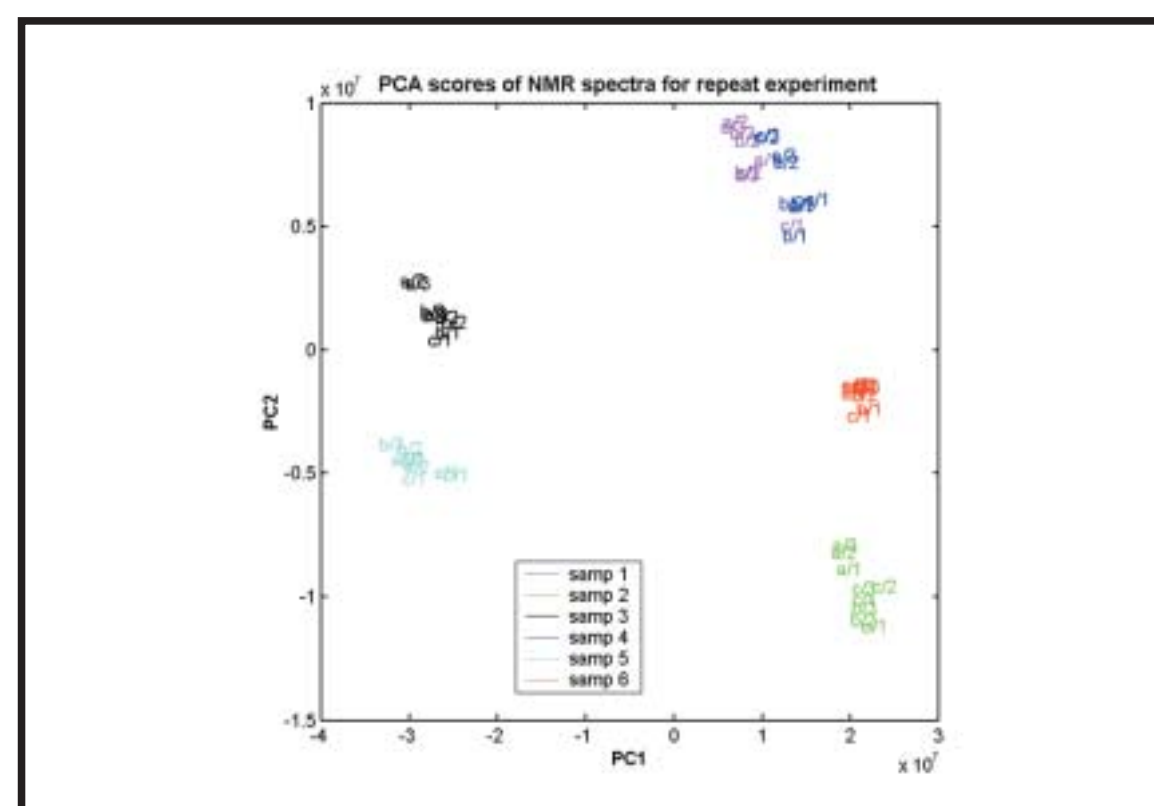


Figure 2 PCA scores plot of NMR spectra for reproducibility study. Each different GM potato sample is shown in a different colour. The letters (a-c) represent the different extractions and the numbers (1-3) the repeated NMR analyses.

Table 1: List of Desiree potatoes used in the analysis

Code	Construct	No. of lines
gen4	Potato GBSS promoter, putative pullulan (W2) gene and CaMV polyA tail	4
gen5	Transformed with 'empty' pBIN19 vector	2
gen6	x2 35S promoter, anti-sense Mal1 gene and CaMV poly tail	7
gen7	x2 35S promoter, sense Mal1 gene and CaMV poly tail	1
gen8	Transformed with 'empty' pBIN19 vector	1
gen9	x2 35S promoter, anti-sense SAMDC gene and polyA tail	6
gen10*	x2 35S promoter, tet-repressor gene, anti-sense SAMDC gene and polyA tail	5
gen11	Transformed with 'empty' pBIN19 vector	1
gen12	None - Tissue culture grown	1

* can be treated as controls for gen9 group

Secondly, the possible occurrence of unintended effects was investigated for a series of transgenic potatoes (cv. Desiree) with the appropriate controls, as listed in Table 1. For this, ¹H spectra of all lines (4 replicates plants per line) were recorded and then analysed by PCA. When using raw spectra, the first PC scores were strongly correlated to the overall NMR intensity and ultimately to the extracts concentration, most likely due to dry matter content differences. Area-normalisation could be used to lessen this effect. The PC score plots showed that the variation between replicate plants could be very different from one case to another: Whilst the replicate plants were tightly clustered in some cases, the spread between replicate plants could also be as large as between lines in other cases. Despite this, clustering of samples from the same construct was observed (see Figure 3); for instance the W2GBSS modification had lower PC1 scores than the two other GM groups. In addition, the control and modified samples for both W2GBSS and SAM35S showed some separation on PC2. Loadings plots and difference spectra revealed wide-ranging differences in metabolite composition.

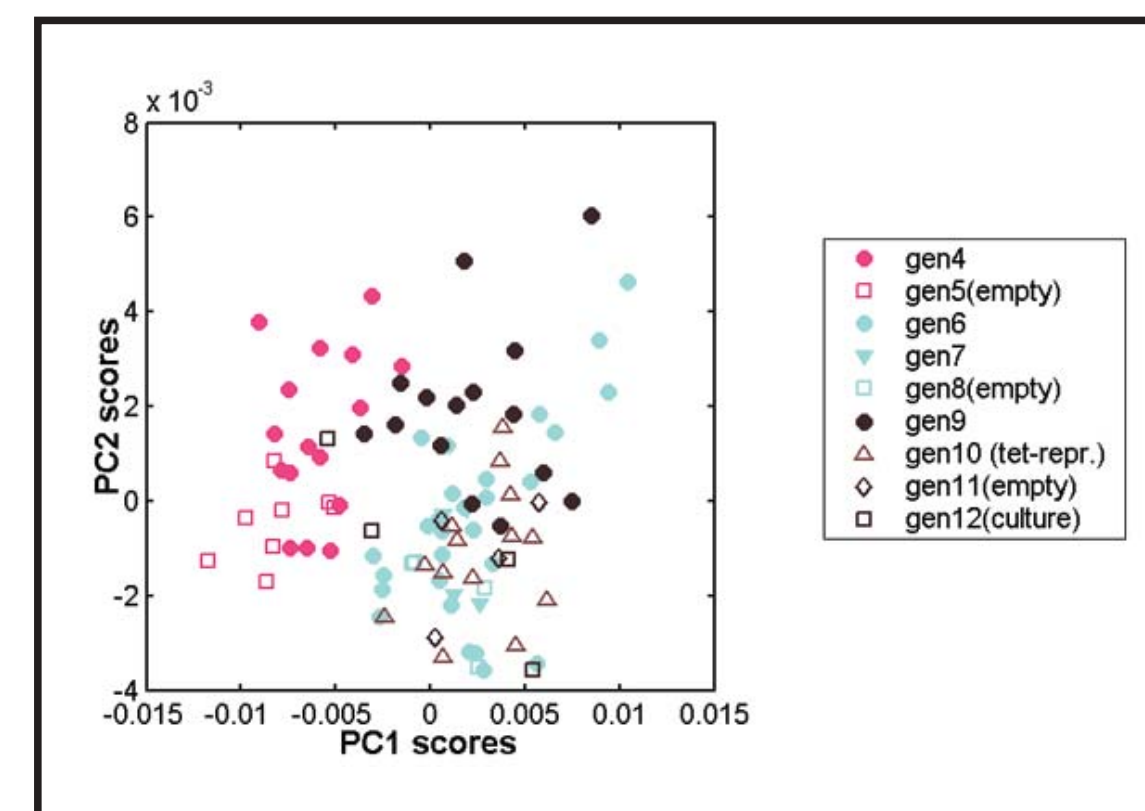


Figure 3 PCA scores plot of the samples listed in Table 1. Each line is shown in a different colour. Open symbols represent control samples and closed ones their genetically modified equivalents.

Thirdly, models were built to quantify glucose, fructose, sucrose, and reducing sugars, using the sugar region of the NMR spectra (3-5.5 ppm). For this, chromatographic data was used as reference. Multivariate partial-least-squares (PLS) regression was employed to enable the utilization of the whole sugar region. The results are shown in Figure 4. This shows one approach to the validation of profiling techniques, a necessary step if they are to be adopted in food safety assessments.

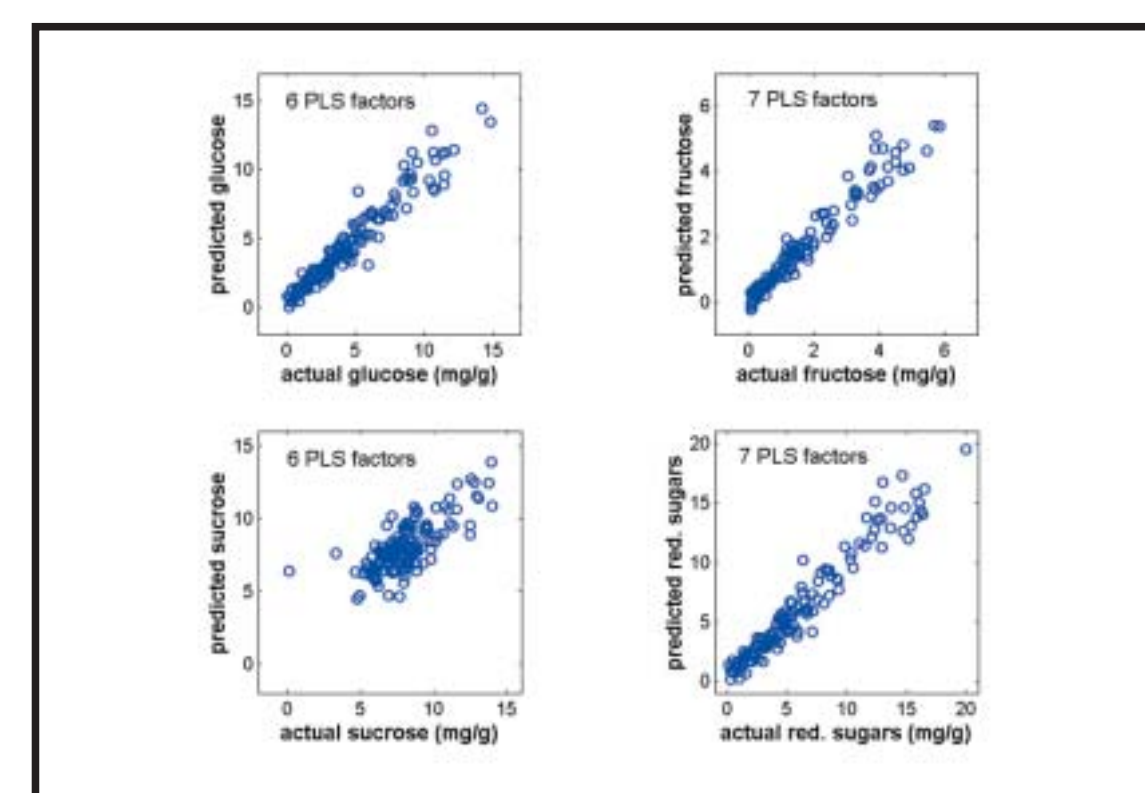


Figure 4 PLS results (predicted versus actual content) for glucose, fructose, sucrose, and total sugars.

Profiling by GC

A GC method was developed for the identification of sugars, amino acids and organic acids. Freeze-dried potato samples were extracted with aqueous alcohol and derivatised using methoxyamine hydrochloride and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). The trimethylsilyl (TMS) derivatives of the metabolites were separated using GC (see Figure 5).

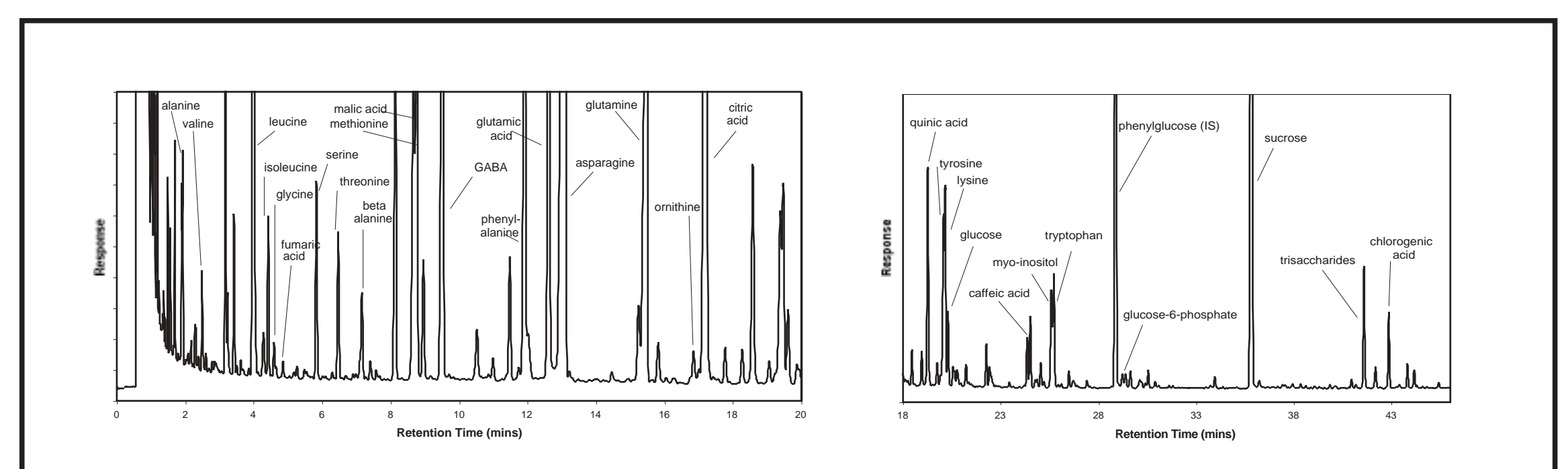


Figure 5 An example of a GC trace for an extracted and derivatised Desiree potato sample, with assignments determined by GC/MS using reference data.

A study was carried out to assess the reproducibility of the derivatisation procedure and the GC instrumentation. A series of 6 GM potato samples were extracted in triplicate, and analysed by GC in duplicate. The resulting chromatographic data was analysed using chemometrics (see Fig 6). The plot shows that each sample (with the exception of 21/2) forms tight clusters, which demonstrates that both the derivatisation procedure and subsequent analysis by GC are reproducible. GC data for the samples listed in Table 1 will be analysed in the same way as the NMR data.

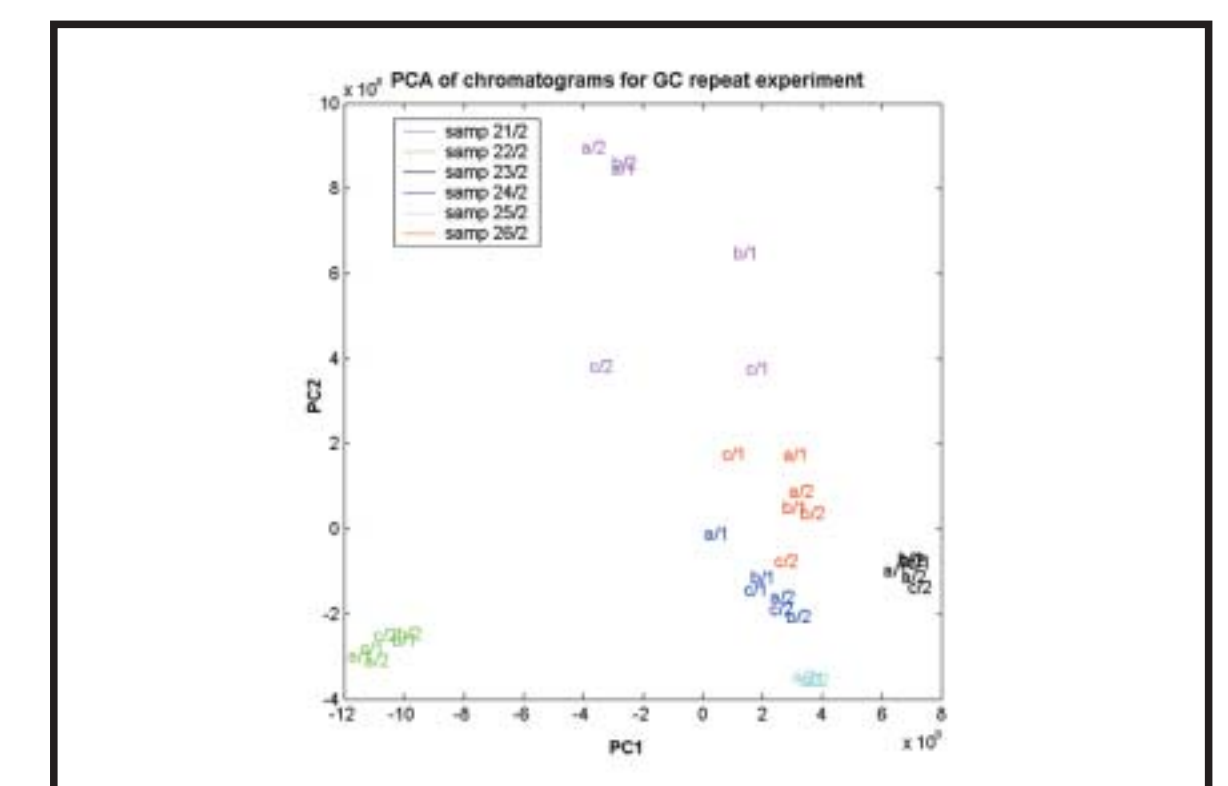


Figure 6 PCA scores plot of chromatograms for reproducibility study. Each different GM potato sample is shown in a different colour. The letters (a-c) represent the different extractions and the numbers (1-2) the repeated GC analyses.