The use of high performance anion exchange chromatography for the detection of counterfeit pharmaceutical products using the excipient content as a marker

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Abstract

The aim of the investigation described here was to examine the differences between genuine and counterfeit pharmaceutical products through the development of an analytical method capable of rigorously identifying the sugar-based excipients. High Performance Anion Exchange Chromatography, coupled to Pulsed Amperometric Detection (HPAEC-PAD), supported by Gas Chromatography-Mass Spectrometry (GC-MS), provided a method for the analysis of the carbohydrate based excipients. The analytical method was able to discriminate between the substitution patterns of a number of monosaccharides derived from commonly used excipients and these were compared for both genuine and counterfeit sildenafil citrate based products. The aim of the project was accomplished: the HPAEC-method was employed to analyse a counterfeit pharmaceutical 'Herbal Viagra'.

Keywords: High Performance Anion Exchange Chromatography; Drug Excipients; Carbohydrates; Sildenafil Citrate.

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Introduction

Millions of people use pharmaceutical products every day, they are used to cure ailments, to treat disease and as preventative measures against diseases. The Pharmaceutical Industry is one of the biggest industries in the world with a net worth estimated at $300-400 billion a year according to the World Health Organisation (WHO)[1]. The continued growth and success of this industry requires consumers to be confident about the supply of safe medicines; however, the supply of counterfeit pharmaceutical products is on the increase. Counterfeit medicines present unprecedented danger to the unknowing consumers and also hinder the growth of genuine products by reducing the revenues needed for their development.

The counterfeit medicine industry is now a global problem. Whilst no current figures are available on the extent of the problem, the WHO estimated the sales of counterfeit medicines to be worth $75 billion a year worldwide in 2010[2]. Pfizer's pharmaceutical security institute define counterfeit medicines as:

'A medicine that has been deliberately and fraudulently produced and/or mislabelled with respect to its identity and/or source to make it appear to be a genuine product.'

This definition includes drugs with no active ingredient, drugs with the wrong active ingredient, drugs with dangerous impurities and drugs that are mislabelled or sold in the wrong packaging [3]. The WHO collected statistics on the different types of counterfeit pharmaceuticals as a bid to raise public awareness, the different types include:

Products without active ingredients – **32.1%;**

Products with incorrect quantities of active ingredients – **20.2%;**

Products with correct quantities of active ingredients but with false packaging – **15.6%;**

Copies of an original product – **1%;**

Products with high levels of impurities and contaminants – **8.5%**[3].

Up to 50% of the medicines available from internet sites which conceal their physical location are believed by the WHO to be counterfeit[4]. The numbers have grown significantly and the figures keep climbing. This is partially due to the variety of different distribution and retail networks used to supply medicines to patients in different parts of the world and the large price difference between branded, generic and counterfeit products. Unfortunately, counterfeit drugs are often very difficult to distinguish from their genuine equivalents and have been found within reputable supply chains: in the UK counterfeit pharmaceuticals have been sold in bulk to unwitting suppliers for use in hospitals and pharmacies. A bulk amount of fake drugs entered the NHS supply chain in 2007, whilst a large number were seized when the error was realised, 25,000 had been distributed to pharmacies and then patients. Of that amount, only 7,000 were ever recovered during a recall of the suspected fakes[5]. This example provides evidence that counterfeit medications and pharmaceutical products aren't just simply a case of buying from dubious people or websites, but involves organised criminal activity.

A wide variety of methods have been used for the detection of counterfeit pharmaceutical products ranging from basic to advanced and specialised techniques; one common attribute many methods share is the detection of the active

pharmaceutical ingredient, also known as API[6]. Thin layer chromatography is a cheap and specific analytical method that is routinely used to detect counterfeit APIs[7]. The technique is advantageous because it is cheap and simple to run as a test, but disadvantages are that reference standards are needed to identify unknowns and the process doesn't quantify the amount of API present.

More advanced techniques that require specific instrumentation can also be applied. The use of high performance liquid chromatography (HPLC)[8] and mass spectrometry (MS)[9] are commonly used instrumental setups for the analysis and comparison of analytes, including pharmaceutical products. In an early publication (2004), both HPLC and MS were used in the discovery of counterfeit Viagra. According to the report published by US Drug Enforcement Agency in their Microgram Bulletin[10], the active ingredient of Viagra, sildenafil citrate, was not found in a counterfeit product but had been replaced by an amphetamine.

One problem with identifying and detecting the API is that its detection does not indicate if it was manufactured appropriately: the material can still be counterfeit material and the API could have been manufactured outside of the quality control systems and approval regulations (FDA/MHRA) required for licensed medication. Alternatively, the product may have been rebranded e.g. a generic product being passed off as a more expensive branded product.

The purpose of this paper is to suggest an alternative method for the detection of counterfeit pharmaceutical products, one which involves the analysis of excipients. Drug excipients are the pharmacologically inactive ingredients that are added to drugs and which often make up the majority of the weight of any dose. The API of a drug is what dictates the mode of action of a drug, for example, a correction of erectile dysfunction. The excipient counterparts are added to influence (normally to enhance) the therapeutic efficacy of the API e.g. to encourage the formulated product to disintegrate and release the active ingredient at a specific point in the body, for example in response to a pH change trigger. The excipients are added during the manufacturing process, some are added so that the API survives the manufacturing process without degradation. Table 1 lists the most common excipient types alongside their usage.

A large number of carbohydrates and carbohydrate based derivatives are used as drug excipients. The advantages of using carbohydrates as excipients are that they are cheap and plentiful. They can be sourced from a combination of natural and synthetic origins. They are easy to make and easy to work with as most are stable solids. They provide both chemical and physical properties to a formulation with minimal interference to the API, but most importantly, minimal interference to the safety of the end user. It is the many unique properties of monosaccharides, disaccharides and polysaccharides that provide drug excipients with their varied and desired attributes.

To date, there are only limited reports[11] of using excipients as markers for determining the authenticity of medicines and none of these use high performance anion exchange chromatography (HPAEC). The work described here seeks to address this gap in knowledge and seeks to determine if analysis of excipient carbohydrates by HPAEC can be used to discriminate between genuine and counterfeit medicines.

Excipient Class	Ingredient	Use
Binders	Cellulose Hydroxypropyl cellulose Lactose	Provides mechanical strength and binds tablet together
Coatings	Hydroxypropyl methylcellulose	Provides protection against environmental factors. Provides smooth surface which produces less friction when swallowed.
Controlled release agents	Hydroxypropyl methylcellulose	Prevents breakdown of tablet until it has reached a specific destination e.g. digestive tract.
Diluents	Lactose Microcrystalline cellulose	Acts as a bulking agent to make tablet more manageable.
Disintegrants	Corscarmellose	Allows the tablet to break-up rapidly when exposed to moisture.
Glidants	Starch Cellulose	Improves flow-ability during tablet manufacture
Lubricants		Ensures tablet remains intact during manufacture

Table 1: List of different types of excipients, the role they play in a formulated product and the carbohydrates which are used to fulfil these objectives.

In order to asses if the analysis of carbohydrate excipients can be used to determine the origin and potential source of formulation of a counterfeit product, a drug was chosen which is a prescribed medication (available only via prescription within the UK) and also where there is a ready alternative but illicit supply (through web-sites). The drug chosen was sildenafil citrate which is sold as a 'Branded Medicine' under the trade name of Viagra and which is manufactured and distributed by Pfizer. Medicines incorporating sildenafil citrate are also available from a range of generic manufacturers under a variety of different trade names. Unfortunately, it is also widely available as a counterfeit pharmaceutical product [12].

The legitimate branded and generic formulations of sildenafil citrate contain a variety of carbohydrate based excipients including: hydroxypropyl methylcellulose, cellulose, lactose, carboxymethyl cellulose and croscarmellose (cross-linked carboxymethyl cellulose). Lactose (Figure 1) is the major carbohydrate constituent of milk and is a disaccharide generated through the condensation of the monosaccharides galactose (Figure 2) and glucose (Figure 3). The most common reason for adding lactose to tablets is as a binder and diluent. Cellulose (Figure 4 R=OH) is a polysaccharide which is composed entirely of anhydroglucose units and is used for a combination of its ability to act as a diluent, glidant, disintegrant and for its adsorbent properties. Carboxymethyl cellulose (Figure 4 R = H or CH_2CO_2H) and hydroxypropyl methylcelluloses (Figure 4 R= H or Me, HOPr, MeOPr) are, as their names suggest, derivatives of cellulose manufactured by alkylation of the anhydroglucose units. In tablet formulations, one of the main reasons for adding carboxymethyl cellulose (CMC) is as a disintegrant. Hydroxypropyl methylcellulose (HPMC) has a more

specific use and is frequently employed as a coating agent and, as such, HPMC is often present at the surface of a tablet where it influences the rate of release of the active ingredient. Finally, croscarmellose is cross-linked carboxymethyl cellulose (XCMC) and is used as a ‘super-disintegrant’.

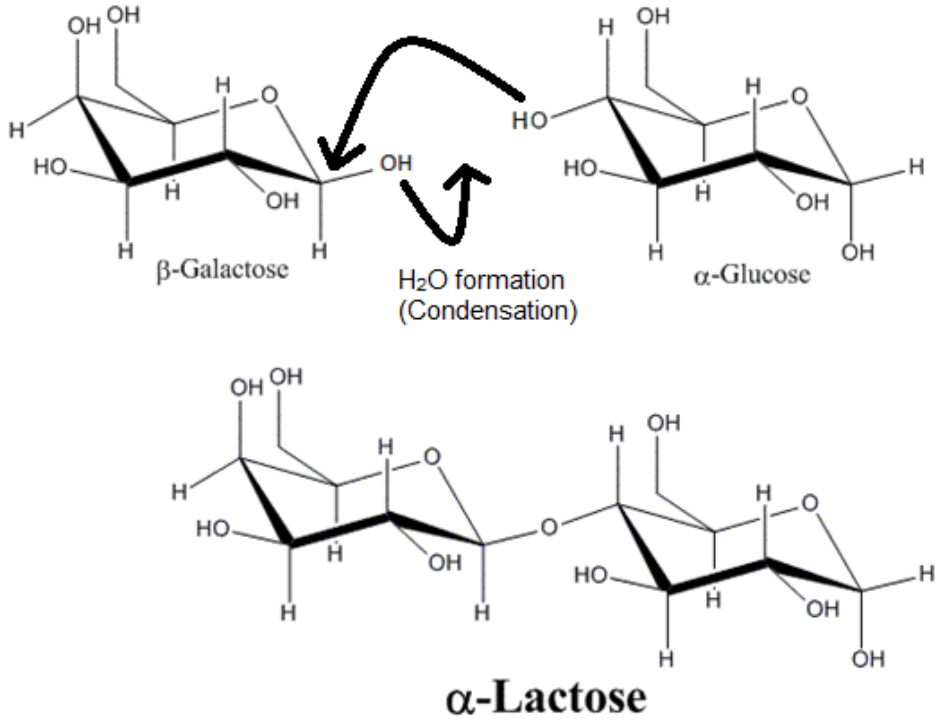

Figure 1. Structure of lactose

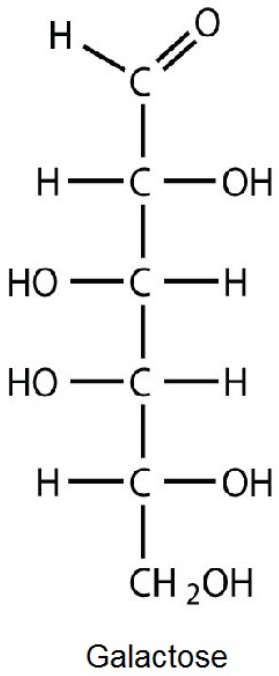

Figure 2. Structure of galactose

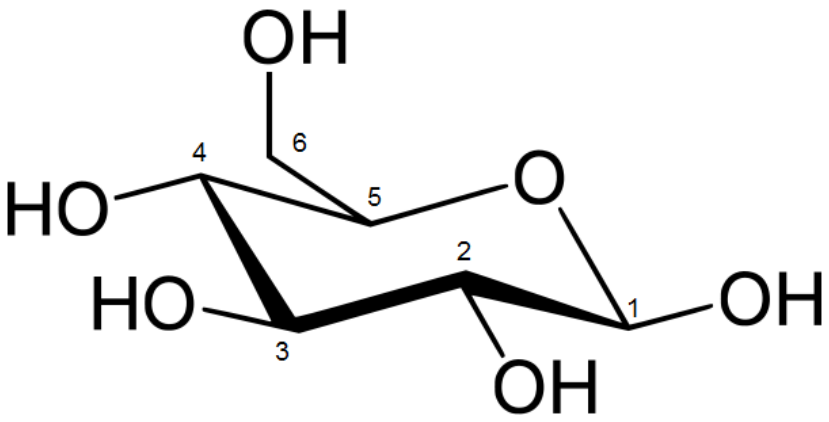

Figure 3. Structure of glucose

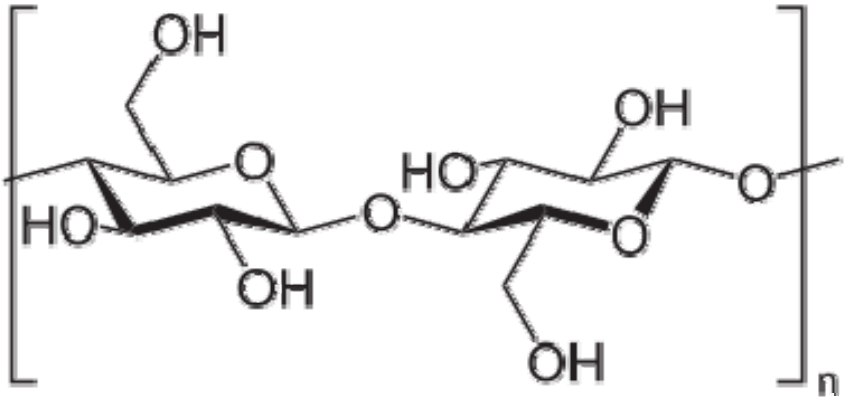

Figure 4 Structure of cellulose

The presence of a variety of different carbohydrates in formulated tablets make them ideal candidates for use in an analytical investigation of their authenticity, with the variation in the structures providing a potential finger-print that can be used to determine the origin of materials.

A number of robust and validated methods have been devised for the analysis of monosaccharides and polysaccharides. The methods most frequently employed include ‘monomer’ analysis13 and ‘linkage’ analysis14. A key step in both procedures is the heating of the carbohydrate component with aqueous acid, the process depolymerises any polysaccharides and oligosaccharides to give either monosaccharides or substituted monosaccharides. Application of acid hydrolysis to the carbohydrate based excipients generates a mixture of monosaccharides from lactose and cellulose and substituted monosaccharides from HPMC, CMC and XCMC.

Experimental

Materials and Sample Preparation

Materials

Unless otherwise stated, general laboratory chemicals were purchased from Aldrich (Poole, UK) and were of analytical grade. Cellulose (Avicel) hydroxypropyl methylcellulose, carboxymethyl cellulose and croscarmellose standards were supplied by (Colorcon Ltd. Kent, UK).

Samples of sildenafil citrate were obtained through prescription from a Pharmacy (West Yorkshire, UK). 'Herbal Sildenafil Citrate' was purchased from a web-based (E-bay) supplier of herbal medicines. For the first set of analyses, tablets (50 mg) were crushed whole in a mortar and pestle. In an additional set of experiments, for those tablets where a blue-coating was visible, the tablet coating was carefully removed using a scalpel and the contents (3 mg) of both the coating and the tablet core were analysed separately.

Acid Hydrolysis

An aqueous solution of trifluoroacetic acid (2 cm3; 2 mol.dm-3) was added to the tablet sample (3.0 mg) in a pressure tube and the tube sealed. The reaction mixture was heated at 120 oC for 2 h. After 2 h, the samples were cooled to room temperature and the cap of the pressure tube was removed. The solution was evaporated to dryness under a constant stream of nitrogen at 65 oC to give monomers which were used directly (HPAEC-PAD analysis- see below) or were converted into their alditol acetates (GC-MS analysis-see below).

Preparation of Alditol Acetates.

The hydrolysed products (3.0 mg) were dissolved in ultra pure water (1.5 cm3) in a pressure tube and sodium borohydride (10 mg) was added. The pressure tube was then sealed and heated at 40 oC for 2 h. After 2 h, the sample was cooled to room temperature and the solution was evaporated to dryness under a constant stream of nitrogen at 65 oC. Once the contents of the flask had evaporated, glacial acetic acid (1 cm3) was added and the solution was left for 5 mins before evaporating to dryness under a constant stream of nitrogen at 65 oC. Once dry, methanol (1 cm3) was added and the solvent was removed by forced evaporation under a stream of nitrogen; this process was repeated a further two times. Finally, a crude mixture of sugar alditols was recovered which was used directly in the next step.

Pyridine (2 cm3) and acetic anhydride (2 cm3) were added to the mixture of sugar alditols in a pressure tube, this was sealed then heated at 100 oC for 2 h. After 2 h, the tube and its contents were cooled to room temperature and the solution evaporated to dryness under a constant stream of nitrogen at 40 oC to give a crude mixture of sugar alditol acetates. The dried alditol acetates were suspended in ultra pure water (5 cm3) and the analytes extracted with chloroform (3 x 5 cm3). The combined organic layer was washed with water (2 cm3) and was dried through the addition of anhydrous sodium sulphate. Once dry, the solution was filtered and the liquid evaporated under a stream of nitrogen. The resulting solid was dissolved in acetone (2 cm3) before being analysed using GC-MS.

Analytical Methods

High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

HPAEC was performed on a Dionex ICS-3000 HPAEC system (Thermo UK Ltd. Hemel Hempstead, UK) operating in isocratic mode and using a PAD detector and employing a quadruple potential waveform. Anion separation was performed on a Dionex CarboPac PA20 column (6 µm particle size, 250 mm length, 3 mm internal diameter and ≤ 10 Å pore size) and a CarboPac PA20 guard column (3 x 150 mm). Unless otherwise stated, analytes were eluted with a NaOH (4mM-10 mM) isocratic

mobile phase at a flow rate of either 0.3 ml.min-1 or 0.45 ml.min-1 and following every fifth analysis, the column was regenerated by eluting the column with NaOH (200 mM) for 15 mins followed by equilibration with the mobile phase for 15 mins before the next injection.

Gas Chromatography-Mass Spectrometry (GC-MS).

GC-MS analysis was performed on an Agilent 7890A GC (Agilent Technologies UK Ltd. Cheshire, UK) equipped with an Agilent 5975B inert XL EI/CI MSD (Agilent Technologies UK Ltd.). Samples, 1 µl split-injection (ratio 10:1) were eluted from an Agilent HP5-MS (30 m x 250 µm-id, 0.25 µm) column (Agilent Technologies UK Ltd.) eluting with helium (1 mL.min-1) and employing electron impact ionisation (70 kev).

The GC-Oven was originally set at 160 °C for 1 min, before rising to 240°C (+3 °C.min-1), and finally being held at 240 °C for 5 mins before returning to 160 oC; the total run time was 33 mins.

Results

Determination of Retention Times of Standards

Analysis of Standards by HPAEC

HPAEC analysis of a series of standards was performed to determine the retention times of the unsubstituted monosaccharides and to establish the substitution patterns for the cellulose derivatives. The HPAEC-PAD results for lactose and cellulose confirmed the retention times for glucose as 6.5 min (NaOH 6mM) and galactose as 6.0 min (NaOH 6mM) respectively. Under the analytical conditions employed in the present study, when croscarmellose and carboxymethyl cellulose were hydrolysed and analysed by HPAEC, a single peak eluting at the same retention time as that of glucose was observed. When HPMC was analysed a complex chromatograph was observed (Figure 5 NaOH 6 mM): six main peaks were visible with the final peak having the same retention time as that of the standard glucose.

Analysis of Standards by GC-MS

GC-MS analysis of the standards gave similar results to the HPAEC analysis. The retention times for per-*O*-acetylglucitol and per-*O*-acetylgalactitol (derived from glucose and galactose respectively) were 12.9 and 13.3 mins. Again, carboxymethyl cellulose and croscarmellose both gave a single peak which eluted at the same time as per-*O*-acetylglucitol. Analysis of HPMC by GC-MS gave a complex pattern of peaks; seven tall well resolved peaks were observed and a large number of smaller peaks were also visible (Figure 6). As was the case for the HPAEC analysis, the final tall peak in the HMPC trace co-eluted with that of the per-*O*-acetylglucitol.

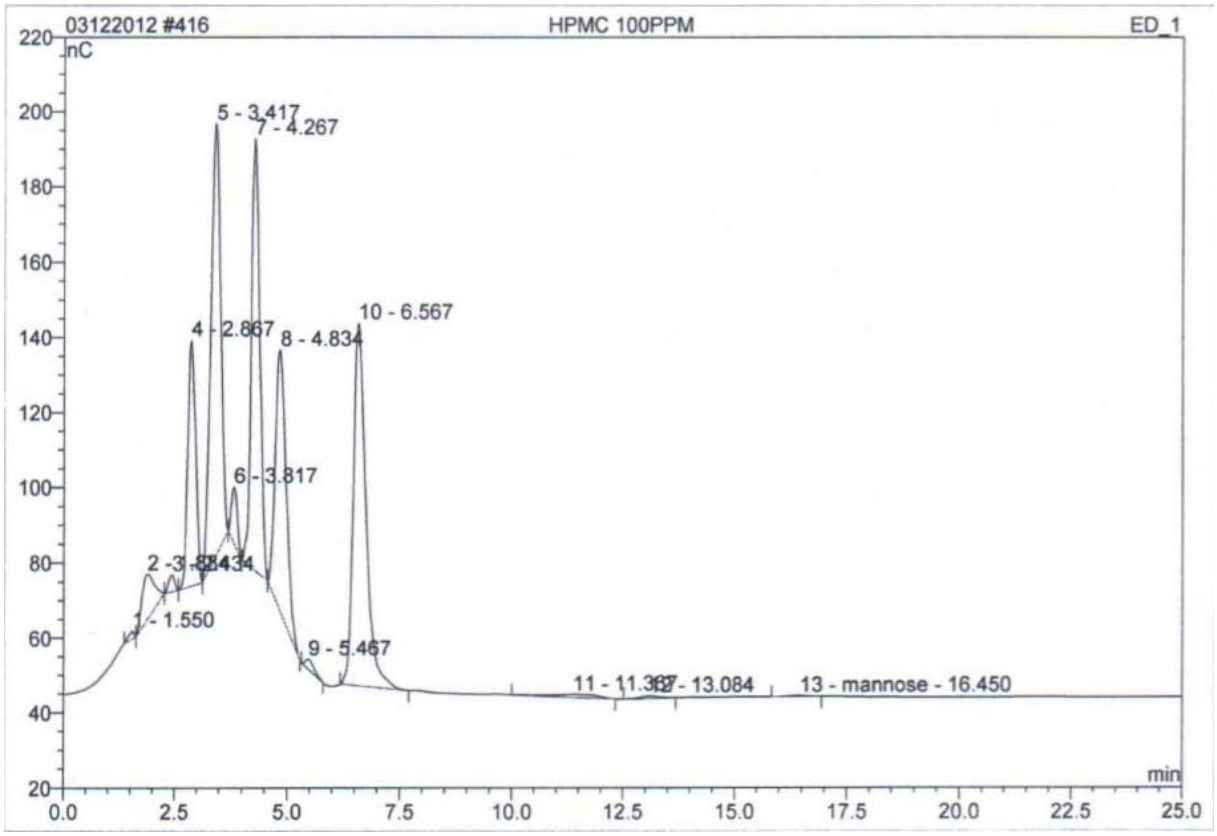

Figure 5. HPAEC-PAD chromatograph for a hydrolysed HPMC standard run with an isocratic mobile phase (NaOH 6 mM)

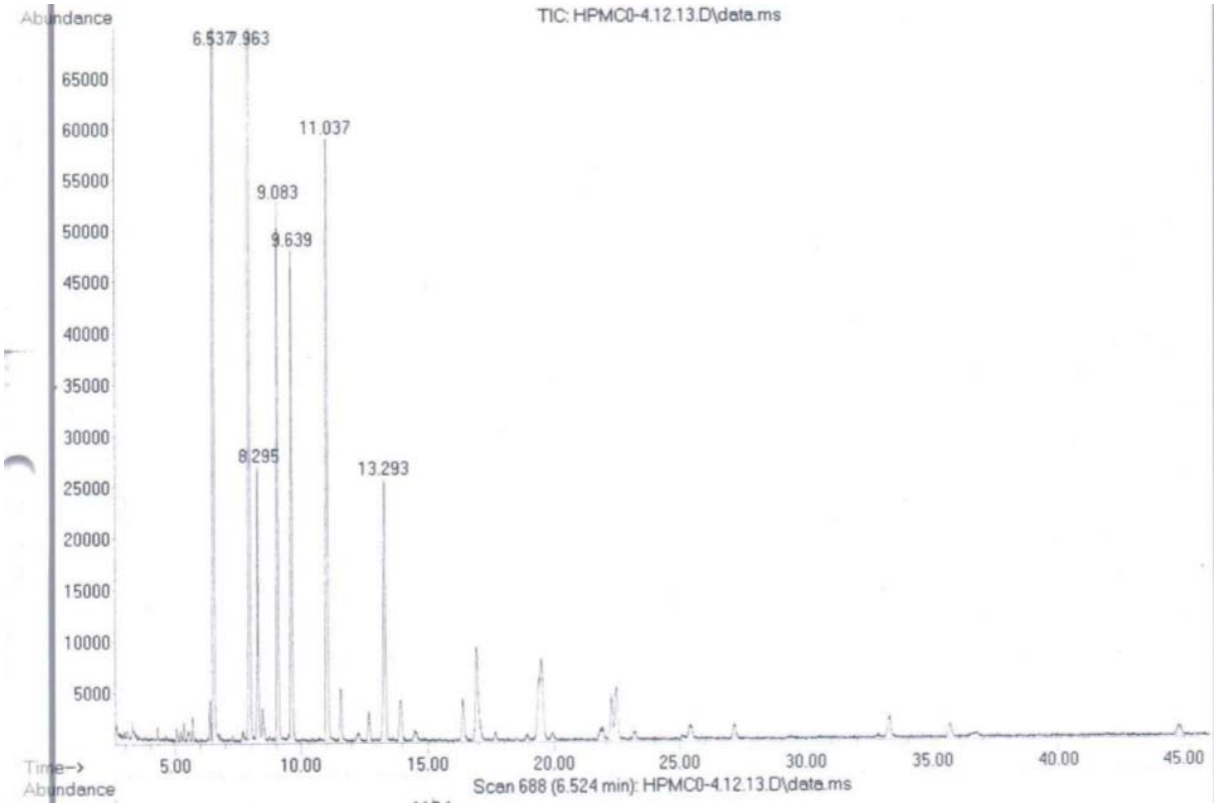

Figure 6. GC-MS chromatograph for a mixture of substituted alditol acetates derived from a HPMC standard

Analysis of the sildenafil citrate tablets

HPAEC-PAD & GC-MS Analysis of the combined coat and core of a sildenafil citrate tablet from a generic product

HPAEC-PAD and GC-MS traces generated from the analysis of the combined coat and core of a representative generic tablet gave two major peaks (Figure 7 (HPAEC – NaOH 4mM) Figure 8 (GC-MS)) corresponding to the free monomers galactose and glucose in the HPAEC & the corresponding per-*O*-acetyl-alditols in the GC-MS.

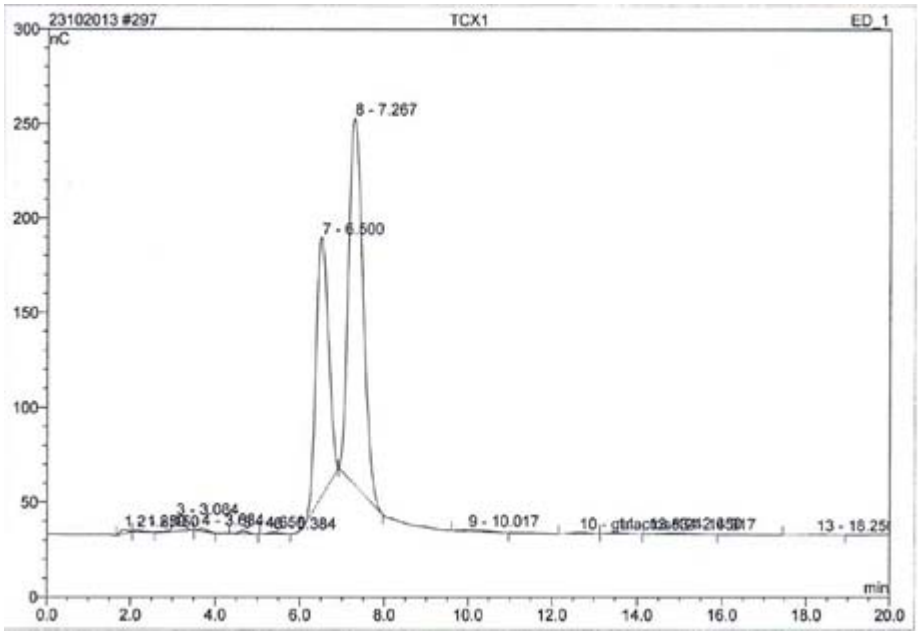

Figure 7. HPAEC-PAD chromatograph for a hydrolysed sample of a generic sildenafil citrate sample including both core and coat run with an isocratic mobile phase (NaOH 4 mM)

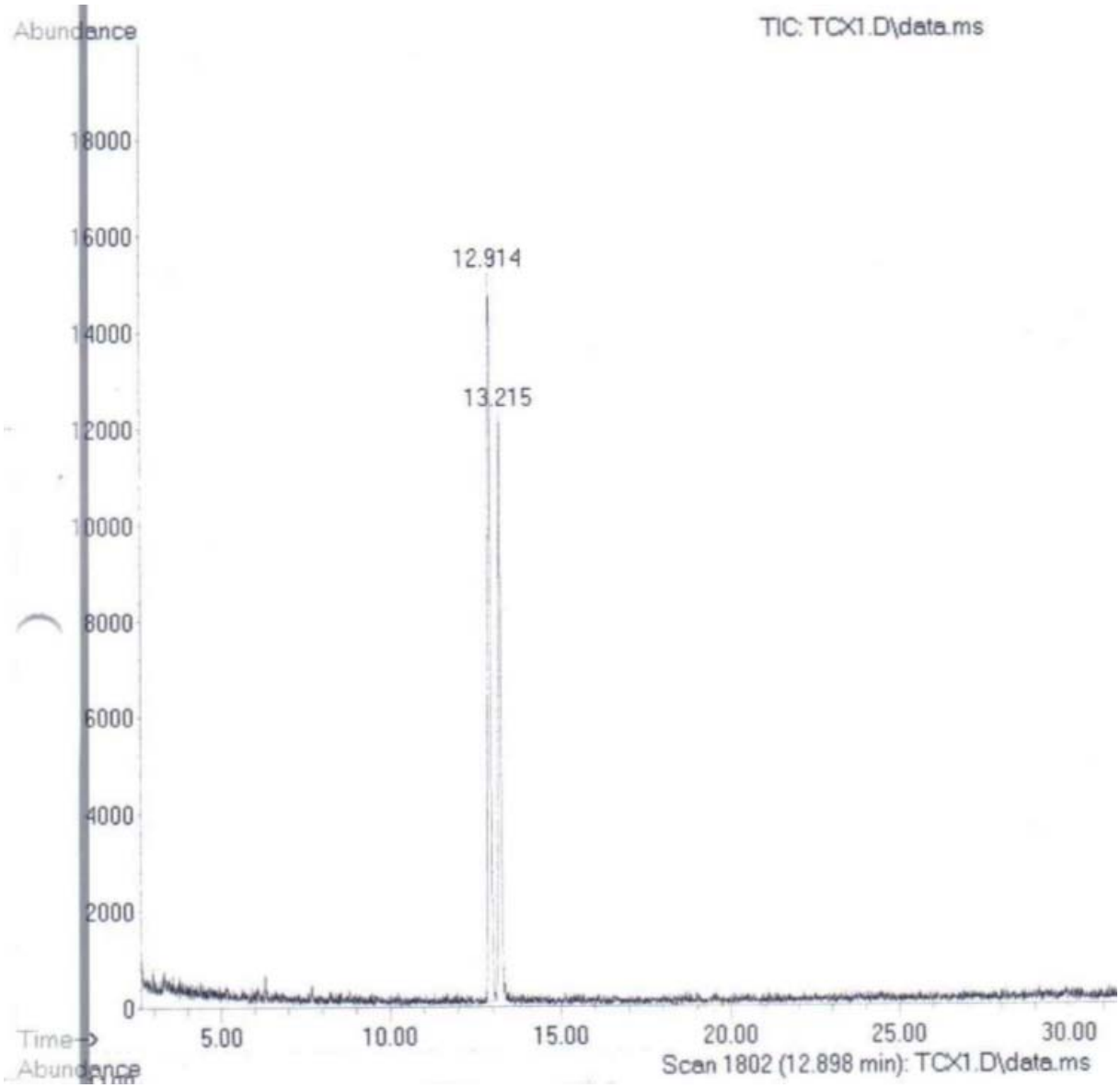

Figure 8. GC-MS chromatograph for a mixture of substituted alditol acetates derived from a generic sildenafil citrate sample including both core and coat

HPAEC-PAD & GC-MS Analysis of the film-coat of sildenafil citrate tablets from a generic manufacturer and the branded product from Pfizer

HPAEC-PAD analysis of the coats from the branded and generic sildenafil citrate tablets produced different chromatographs. The branded (Pfizer) tablet gave a complex HPAEC-trace (Figure 9 – NaOH 5 mM) which included two major peaks with retention times similar to those of glucose and galactose at the end of the trace and a number of medium-sized over-lapping peaks eluting in front of the unsubstituted monosaccharides, which are reminiscent of those observed in the HPMC samples. A similar pattern was observed in the GC-MS trace (Figure 10).

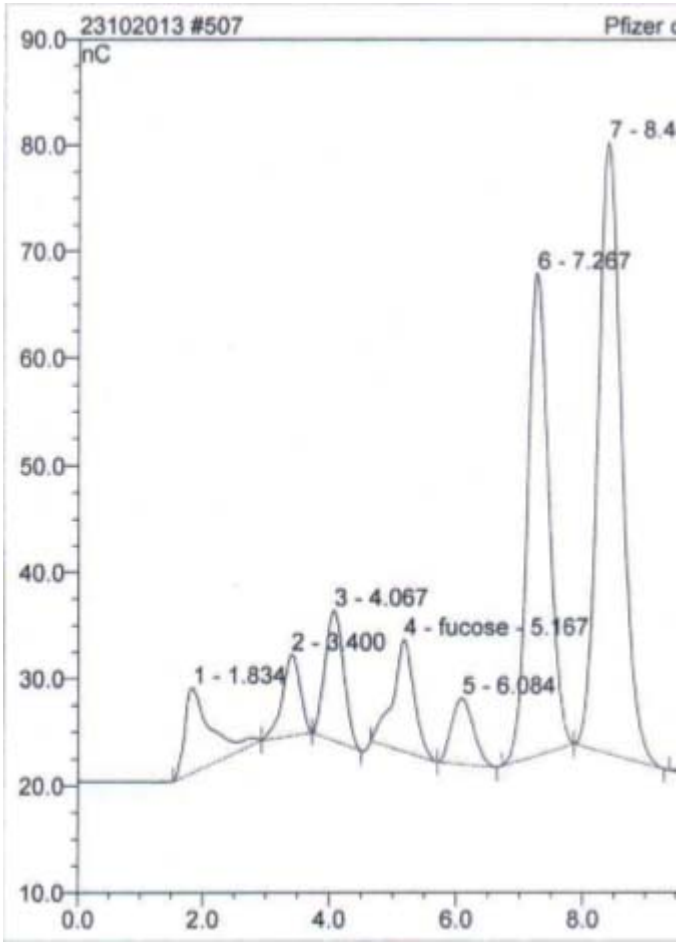

Figure 9. HPAEC-PAD chromatogram for a hydrolysed sample of the film coating of a branded sildenafil citrate sample with an isocratic mobile phase (NaOH 5 mM)

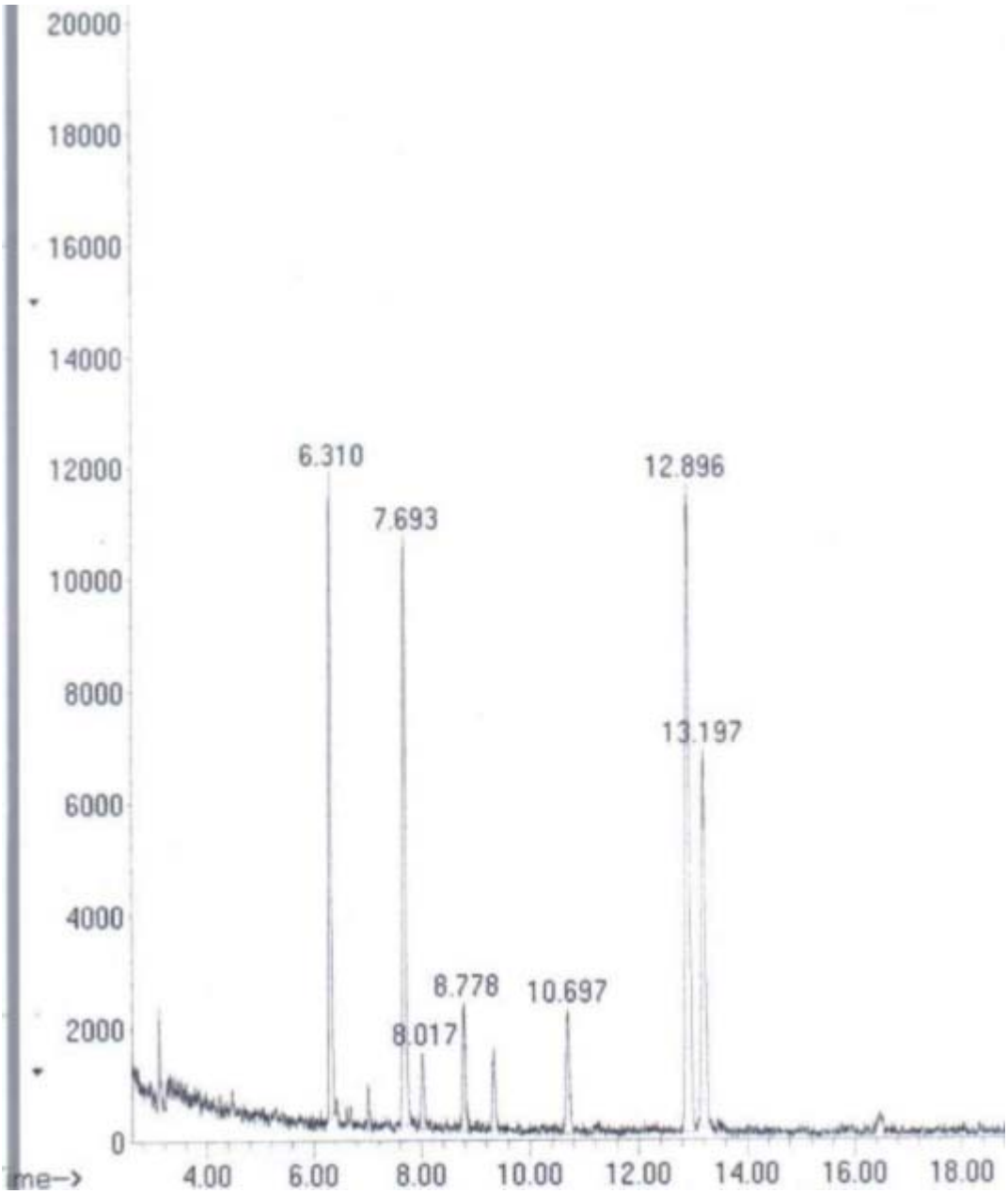

Figure 10. GC-MS chromatograph for a mixture of substituted alditol acetates derived from a sample of the film coating of a branded sildenafil citrate sample.

For the generic formulation the HPAEC trace of the coat has a similar overall profile: two major peaks corresponding to galactose and glucose appear at the end of the trace and these follow a number of much smaller peaks which, again, are reminiscent of those observed in the HPMC samples (Figure 11); the same is true for the GC-MS profile (Figure 12).

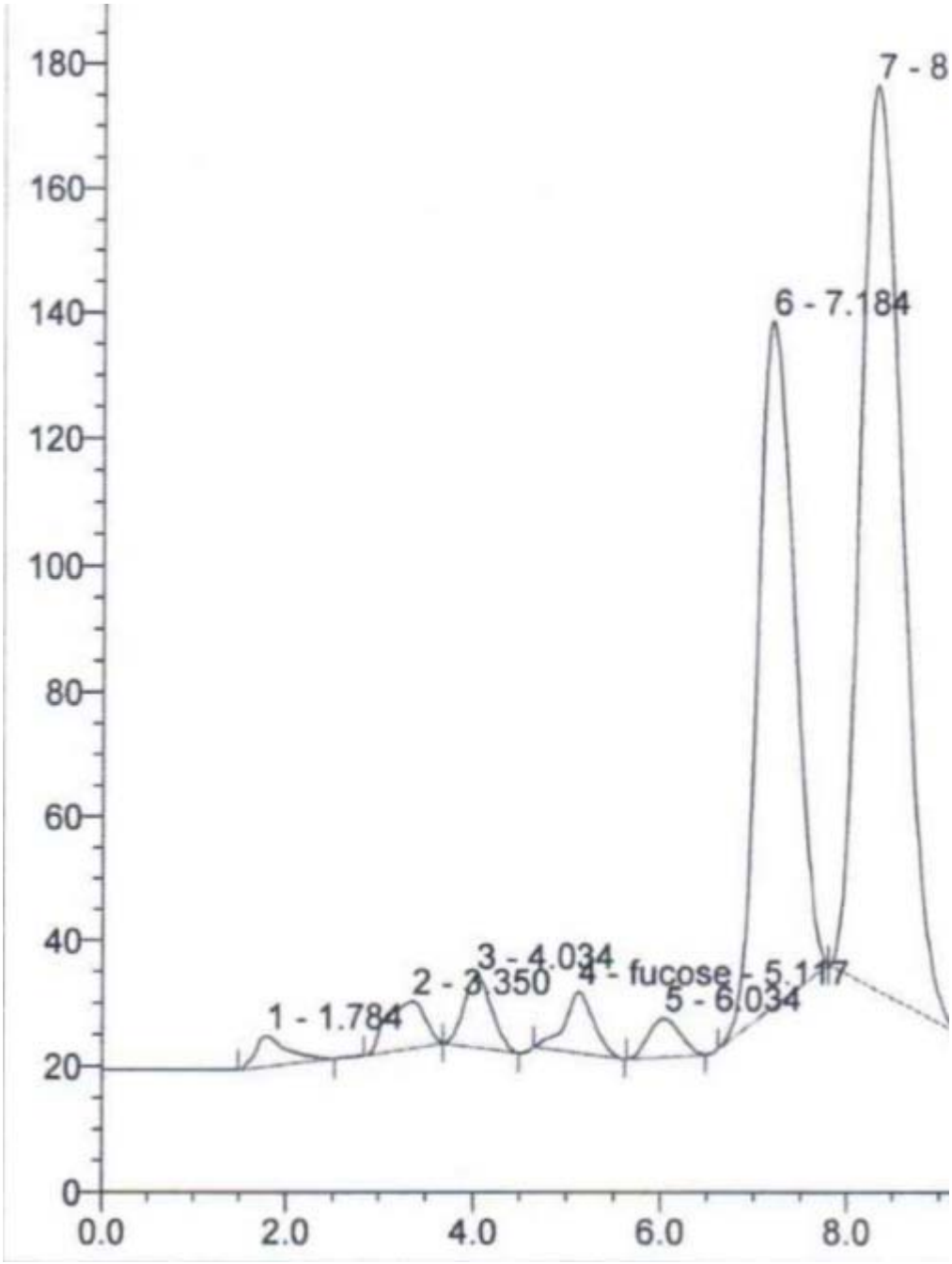

Figure 11. HPAEC-PAD chromatographfor a hydrolysed sample the film coating of a generic sildenafil citrate sample with an isocratic mobile phase (NaOH 5 mM)

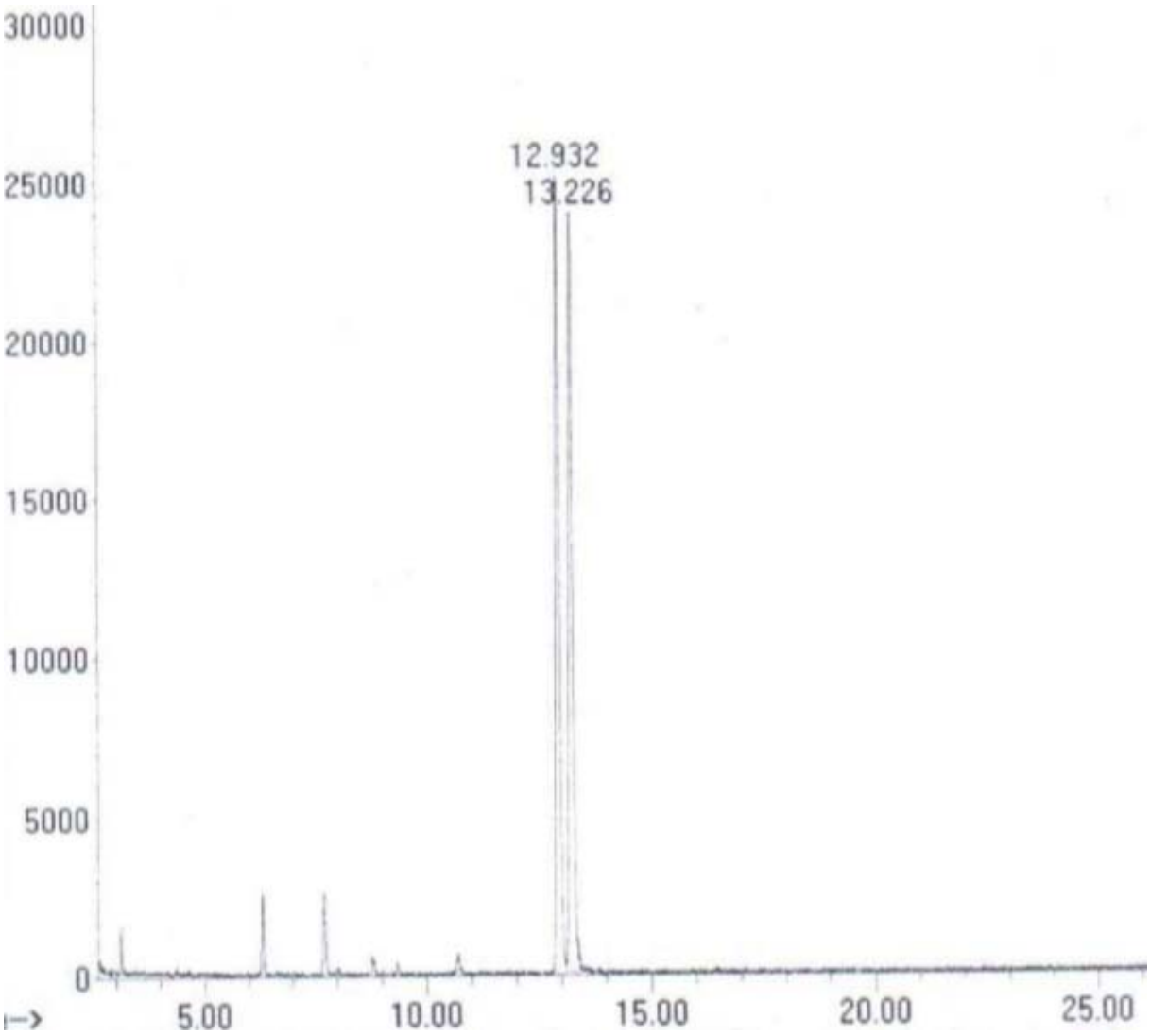

Figure 12. GC-MS chromatograph for a mixture of substituted alditol acetates derived from a sample of the film coating of a generic sildenafil citrate sample.

HPAEC-PAD Analysis of the isolated coat of a suspect counterfeit product 'Herbal Sildenafil Citrate'.

For the counterfeit product, the HPAEC trace of the coat has a different profile to that obtained with the branded and generic products: one major peak corresponding to galactose follows a number of small sized peaks (Figure 13). Again, a very similar profile was observed in the GC-MS analysis (Figure 14).

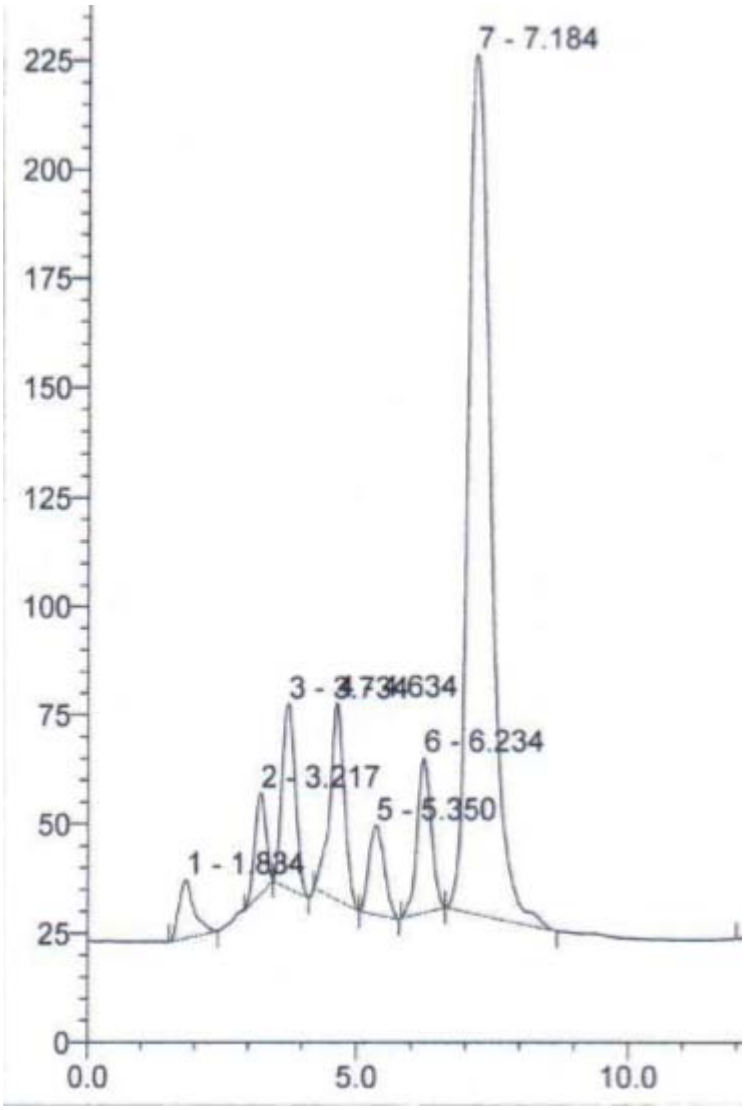

Figure 13.HPAEC-PAD chromatograph for a hydrolysed sample the film coating of a ‘herbal sildenafil citrate sample’ with an isocratic mobile phase (NaOH 5 mM)

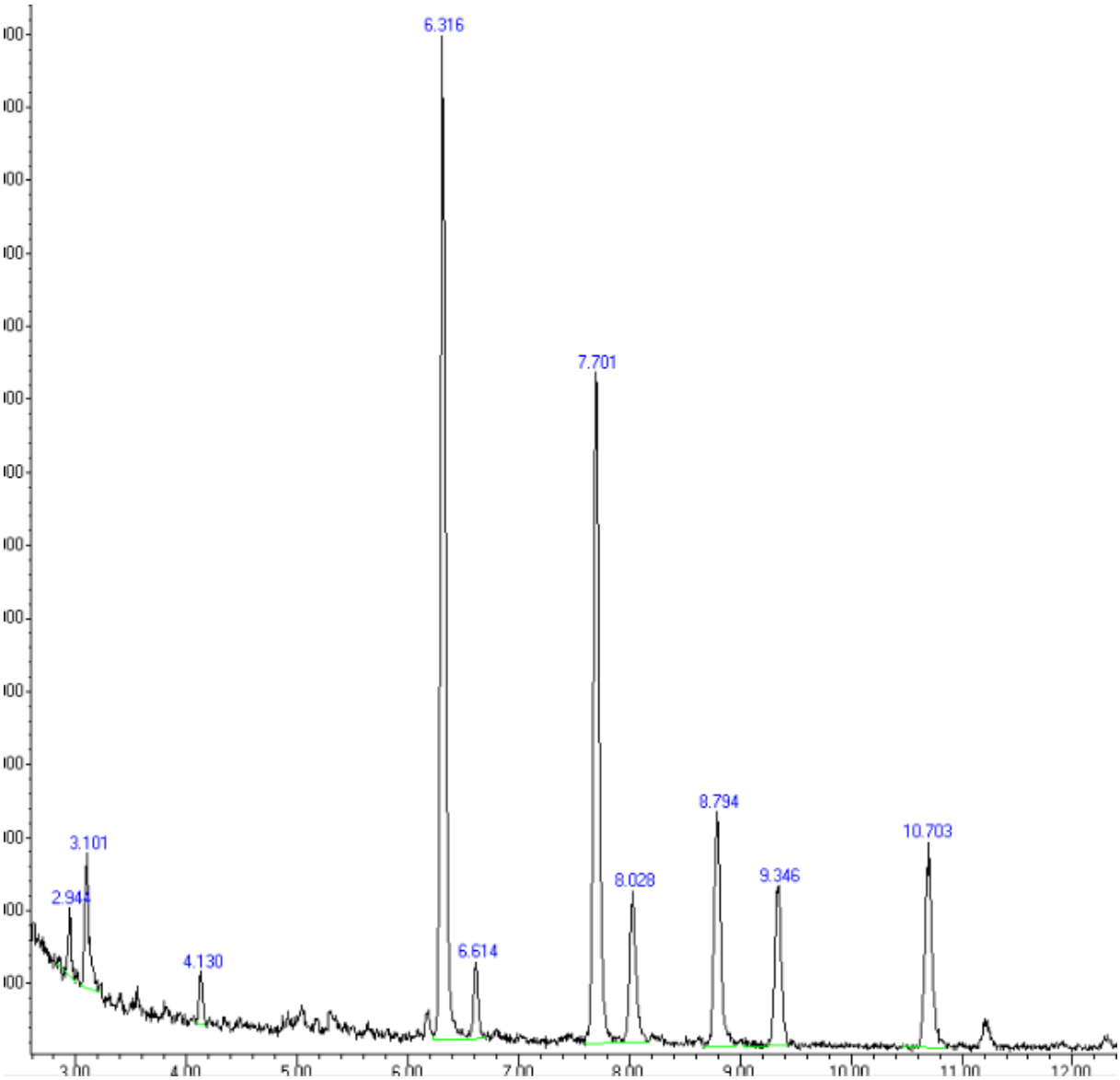

Figure 14 GC-MS chromatograph for a mixture of substituted alditol acetates derived from a sample of the film coating of a 'herbal sildenafil citrate sample.

Discussion

Analysis of standards, including lactose, cellulose, croscarmellose, carboxymethyl cellulose and hydroxypropyl methylcellulose

In order to identify the carbohydrate materials that make up the excipients in the sildenafil citrate tablets, analytical methods had to be chosen which were able to separate the analytes and which also had the appropriate sensitivity to allow quantification of the peaks of interest. The two methods that were chosen were HPAEC-PAD and GC-MS.

HPAEC-PAD has many advantages over more conventional carbohydrate analysis techniques: it is quick to use, little sample preparation is required prior to analysis and it can detect very low levels of sugars. Historically, GC-MS has been the technique of choice for analysis of carbohydrates[15] and this is because of the ability of the technique to resolve peaks for a large number of different sugars in a single

analysis. However, the general procedures required to derivatise sugars before they can be analysed by GC-MS are lengthy and take several days to perform.

Development of an analytical method based on HPAEC

In the development of an analytical procedure for the analysis of excipients based on HPAEC-PAD, the first task was to establish chromatographic conditions that would separate simple monosaccharides and which could also separate the substituted monosaccharides that would be generated through the hydrolysis of the cellulose derivatives. A number of authors have reported the use of HPAEC-PAD[16] for the separation of monosaccharides derived from plant and microbial sources. As a starting point, conditions similar to those reported by the instrument manufacturers were employed and involved eluting the analytes through a CarboPac PA20 column using sodium hydroxide as a mobile phase.

Using a mobile phase composed of aqueous sodium hydroxide (6 mM), the hydrolysis product of lactose gave two closely eluting but base-line resolved peaks with retention times identical to those of glucose and galactose standards. Croscarmellose and carboxymethyl cellulose are prepared by a reaction which carboxymethylates cellulose and, depending on the degree of substitution, will have varying levels of carboxymethyl-substitution at C6 (**4** R6= CH_2CO_2Na, R_2 & R_3 = H). In croscarmellose, in a subsequent condensation step, ester links are formed between a number of the carboxymethyl groups and a fraction of the hydroxyl groups on neighbouring cellulose chains. The acid hydrolysis of both polysaccharides would be expected to give a mixture of glucose, from unsubstituted anhydroglucoses, and carboxymethylated-glucoses. However, under the mildly basic conditions used to elute the anion column that were employed in the present study any carboxymethylated-glucoses would not be expected to be released from the CarboPac PA20 column. Indeed this was observed, when the analysis was applied to croscarmellose and carboxymethyl cellulose only a single peak was observed which had a retention time the same as that of glucose.

When the hydrolysed hydroxypropyl methylcellulose (HPMC) was analysed a large number of peaks were observed on the chromatogram. HPMC is formed by two successive alkylations of cellulose: in the first propylene oxide is used to add hydroxypropyl groups to a proportion of the C6, C3 & C2 positions of the cellulose backbone; in the second a fraction of the remaining free hydroxyl groups i.e. at C6, C3, C2 and the newly added hydroxypropyl groups are methylated. The overall process can generate a large number of different substitution patterns for the anhydroglucose units (**4**, R2, R3, R6 = H, Me, HOPr or MeOPr). Due to steric constraints and because of the heterogeneous nature of the alkylation reactions, not all substitution patterns will be observed and some patterns will be favoured over others. The variability in the substitution patterns of HPMC which are generated during its manufacture makes this polymer an ideal candidate for use in identifying the origin of a tablet formulation containing HPMC.

When the HPMC standards were analysed by HPAEC-PAD using aqueous sodium hydroxide as an eluent, a set of overlapping peaks was observed. Inspection of the chromatogram showed that six large peaks were visible (Figure 5) and a number of smaller peaks were also present. Given that a large number of differently substituted monosaccharides were expected, the observation of a relatively small number of peaks was surprising. At first it was thought that groups or sets of analytes containing the same number or types of substituents were co-eluting and attempts were made to increase the retention times of peaks in order to try and resolve analytes with

similar retention times. A number of attempts were made to improve the resolution of the substituted monosaccharides by varying the pH of the mobile phase (4 mM-10 mM). Unfortunately, whilst increased retention times were observed at lower sodium hydroxide concentrations, the resolution between peaks did not change.

The use of aqueous sodium hydroxide as the eluent, at low milimolar concentrations, produces a mobile phase with a pH in the range of 12-13. At such high pHs, monosaccharides are ionized to varying degrees and they can be separated by anion exchange[17]. The order of elution for monosaccharides is dependent on the relationship between the pKa of the analyte and the pH of the eluent. The higher the pKa of a given analyte the earlier it will elute from the column and vice versa. Glucose has a pKa value of 12.28 whilst galactose has a pKa value of 12.39 and therefore galactose should elute before glucose and this was observed. This relationship between time of elution from the column and the pKa can be explained as a function of monosaccharide structure and the amount of hydroxyl (OH) groups on each structure. Generally speaking, the more acidic a molecule is the more hydroxyl groups it will contain and these more acidic sugars will bond tighter to the column than sugars that are less acidic[20]. This suggests that replacement of the hydroxyl groups with ether linkages should reduce the acidity and increase the pKa of the monosaccharides. However, the amount of hydroxyl groups is not the only reason a sugar is acidic, some hydroxyl groups provide more acidity due to their position on the carbon back-bone. Bruggink[18] revealed that the hydroxyl group linked to the number one position on the pyranose ring had the biggest impact on a monosaccharide's acidity. Thus, the loss of this hydroxyl group would reduce the elution time more so than the loss of any other hydroxyl group. The substituted monosaccharides generated from HPMC will all possess a hydroxyl-group at C1 and C4 due to the presence of the (1-4)-glycosidic linkage in cellulose, however, they will have varying levels of alkylation at C2, C3 and C6. The presence of the electron releasing alkyl groups should destabilise anion formation at C1 and consequently increase the pKa of the monosaccharides. This is confirmed by the elution of the substituted-monosaccharides of HPMC before the parent monosaccharide glucose.

When the samples were analysed using GC-MS the chromatograms showed well resolved peaks. For the HPMC standard seven peaks having a peak area on the chromatograph representing more than 10% of that of the total were observed (Figure 6). As was the case in the HPAEC system, the acetylated alditols derived from the substituted monosaccharides eluted in advance of those generated from the free monosaccharides. In the GC-MS traces there is little evidence for overlapping peaks and this suggests that the only a specific sub-set of the substituted monosaccharides are present. The major advantage of the GC-MS system is that through the acquisition of mass spectral data the extent of methyl, hydroxypropyl and methoxypropyl-substitution of the different analytes could be determined. From inspection of the mass spectra (data not shown) it was clear that whilst the major peaks contained methyl-substitution there was little evidence for the presence of either hydroxypropyl or methoxypropyl substituents. The failure to observe the hydroxypropyl and methoxypropyl-substitution in the GC-MS (and more than likely also in the HPAEC-studies) suggests that hydrolysis of the glycosyl propyl ether link is occurring during monomer analysis. In order to confirm that the major peaks were methylated-glucoses a sample of methylcyclodextrin was hydrolysed and analysed by HPAEC and the acetylated alditols by GC-MS (data not shown). The retention times of the peaks from the HPAEC and GC were very similar and the mass spectra were identical to those observed for HPMC, only the ratios of the different peaks were different.

Whilst our failure to observe the complete range of different substitution patterns reduces the amount of information available, it does have the advantage of greatly simplifying the chromatograms that are produced.

Analysis of sildenafil citrate tablets

Analysis of the combined tablet from the generic sildenafil citrate formulations using HPAEC-PAD and GC-MS

The information leaflet supplied with the generic sildenafil citrate tablet identified cellulose, lactose and croscarmellose as the carbohydrate based excipients that were added in the tablet core and that HPMC was present in the film coating. Combined analysis of both the coat and core identified galactose and glucose and as such the analysis only provided information about the excipients in the tablet core. It is clear that the core components, cellulose, lactose and croscarmellose hydrolyse to give glucose and galactose, which are dominant signals in both the HPAEC-PAD and the GC-MS traces. The relatively small amount of HPMC from the film-coat was not visible.

HPAEC-PAD & GC-MS analysis of the film-coat of sildenafil citrate tablets from a generic manufacturer and the branded product from Pfizer.

As it was suspected that HPMC would provide the most information about the origin of different tablets, it was decided to remove the film coating from the tablets and to analyse these separately. Film coats were carefully removed using a scalpel taking care to avoid contamination of the film by core material. When the hydrolysis products derived from the film were analysed by HPAEC both the generic product and the branded product gave distinctive chromatographs. The excipients used by manufacturers in the film coating vary: the generic manufacturer lists HPMC as the only carbohydrate based excipient present in the coat whereas Pfizer lists both lactose and HPMC as being present. When the HPAEC and GC-MS traces were analysed there was evidence for both galactose and glucose in both samples (7.2 and 8.3 min peaks in the HPAEC and 13.2 and 12.9 in the GC Figs 7-10). It was not clear why galactose, which can only be derived from lactose, was visible in the film-coating from the generic product. The ratio of glucose to galactose was very similar in the two samples and this was to be expected if these were primarily derived from lactose.

The chromatography traces also provided strong evidence for the presence of substituted monosaccharides which were more than likely derived from the HPMC. The number of peaks (excluding glucose and galactose) was the same for each sample; the only difference was in the relative abundance of signals within each sample and the difference in relative abundance of particular signals between the different samples.

HPAEC-PAD Analysis of the isolated coat of a suspect counterfeit product 'Herbal Sildenafil Citrate'

The HPAEC-chromatograph obtained for the analytes released during hydrolysis of the film-coat from the herbal sildenafil citrate tablet contained one large peak which co-eluted with galactose. A number of smaller peaks which corresponded to those expected for the substituted monosaccharides derived from HPMC were visible in front of the galactose peaks. Inspection of the chromatogram clearly demonstrates

that the 'Herbal Sildenafil' has been manufactured using coating technology employing HPMC. It is clear that the different HPAEC profiles of the 'herbal' product, the branded product and the generic products suggest that the material has been manufactured and formulated independently and is not a misplaced product that has been diverted from a legitimate manufacturing operation. It is worth noting that NMR analysis was used to determine that this product, which was labelled as 'Herbal Viagra', contained sildenafil citrate as its API, a ^{1}H NMR spectrum of a chloroform extract of the tablet core confirmed the identity of the API.

Conclusion

To summarise, the aim of this work was to investigate the differences and similarities of excipients within pharmaceutical products and to create a method by which counterfeit products could be detected. The success of the project has largely been the development of a technique employing HPAEC for the development of a 'fingerprint' for formulations containing HPMC as excipients.

In future work, it is proposed that a library should be developed of HPMC 'finger-prints' for a wide range of commercial excipients and that the library could then be used to routinely test the integrity of supply chains of legitimate branded pharmaceutical and generic products .

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