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ESTIMATION OF NEUTRAL SUGARS AND SUGAR ALCOHOLS IN BIOLOGICAL FLUIDS BY GAS–LIQUID CHROMATOGRAPHY

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CONTENTS

1. INTRODUCTION

Many sugars and sugar alcohols occur naturally in biological fluids, often in low concentrations. Since more than 40 such compounds have been demonstrated in human urine[1] and 21 in human seminal plasma[2], it is necessary to use specific and sensitive techniques for their detection and measurement. Gas–liquid chromatography (GLC) is particularly suitable for such studies since it combines great sensitivity with high resolution. The purpose of the present review is to survey the use of GLC for the measurement of free sugars and sugar alcohols in biological fluids. Its use in the structural analysis of the carbohydrate components of glycolipids and glycoproteins has been considered in previous reviews[3–5].

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2. DERIVATIVE FORMATION

Sugars and sugar alcohols are non-volatile compounds which cannot be analysed by GLC unless volatile derivatives are first formed. Many derivatisation procedures have been described which involve methylation, acylation, trimethylsilylation or butylboronate formation. In all these procedures several chromatographic peaks may result from a single sugar due to α- and β-anomers and ring isomers occurring, each of which may form a separate derivative[6]. Thus analysis of samples containing several sugars may produce a very complex chromatogram. Patterns characteristic of an individual sugar may be an advantage when investigaing the composition of complex saccharides such as glycoproteins, since their recognition will increase the degree of confidence with which a single sugar may be identified[5]. However, the formation of multiple derivatives has several disadvantages in quantitative analysis of free sugars. Sensitivity will be reduced, since the amount needed to give a minimum detectable response will be greater if several rather than single peaks are formed. Chromatograms may be crowded, with overlapping and coincidence of peaks. In addition, inaccuracies would result if variations occurred in the proportions of each isomer formed within an analytical series. To overcome these problems, additional derivatisations have been described which are used in conjunction with acetylation and trimethylsilylation in order to reduce the incidence of multiple products. These additional procedures are alditol, oxime, methyloxime and aldononitrile formation.

2.1. Methyl derivatives

Before the introduction of GLC sugars were separated as methylglycosides. It is therefore not surprising that in the first report of GLC of sugars[7] anomers of D-xylose, L-arabinose, D-glucose, D-mannose and D-galactose were separated as methyl derivatives. The anomeric forms of a single monosaccharide have also been resolved using this technique[8]. Kircher[8] also showed that derivatised sucrose was sufficiently volatile for chromatography and in 1962 Bishop[9] demonstrated that mixtures of fully methylated disaccharides could be resolved. Methylation has also been used to separate isomeric sugar alcohols[10].

Early methods of methylation were cumbersome, but simplified procedures have now been described which are more suitable for the analysis of large numbers of small specimens[11]. This type of derivatisation, in which sugars are methylated by methylsulphinylmethide carbion generated from sodium hydride and dimethyl sulphoxide has been used in the separation and quantitation of disaccharides occurring in faecal homogenates[12].

2.2. Acyl derivatives

2.2.1. Acetyl derivatives. The separation of sugar alcohols and monosaccharides as acetyl derivatives was first described by Gunner *et al.*[13] who, in addition to separating sugar alcohols of differing molecular weight, found that isomeric polyols could be resolved. Anomeric glycose acetates gave separate peaks, and derivatives of epimers had different retention times. The technique was extended to the separation of disaccharides by using more thermostable stationary phases which were coated in thinner films on the inert support[14,15].

To eliminate the formation of multiple derivatives from a single sugar the carbonyl group of monosaccharides can be reduced with sodium borohydride to form the corresponding sugar alcohols[16]. These are then acetylated before being injected onto the chromatograph. This technique has been extended to the analysis of complex carbohydrates from soil samples by Oades[17] who described derivatisation procedures suitable for the routine handling of specimens. Samples were dried and acetylated by adding acetic anhydride and pyridine (50:50, v/v), then standing overnight at room temperature. This procedure was adapted to the analysis of sugar alcohols in the serum and urine of uraemic and diabetic patients by Pitkänen[18], sodium borohydride reduction being omitted. Since glucose was not separated from galactitol or sorbitol it was removed by initial incubation of the sample with glucose oxidase, the resulting gluconate being adsorbed with an ion-exchange resin. This type of procedure has been used to study sugar alcohols in cerebrospinal fluid[19,20] and adapted to quantify polyols in human lens tissue from a patient with galactosaemia[21]. Reduction followed by acetylation has been used to quantify disaccharides in biological fluids[22]. There are however disadvantages to this type of method being used to estimate monosaccharide levels: naturally occurring polyols would cause errors in quantitation, and two monosaccharides may give rise to the same sugar alcohol (*e.g.*, arabinose and lyxose).

An alternative approach is the preliminary conversion of aldoses to the corresponding methyloxime or aldononitrile derivative, followed by acetylation of free hydroxyl groups. The incidence of multiple derivatives from a single sugar is reduced since α/β-anomerisation does not occur. This is because these additional procedures involve reactions with the C-1 position of aldoses. Since they are fully reduced, sugar alcohols do not form methyloxime or aldononitrile derivatives. Derivatisation with methoxylamine hydrochloride, followed by acetylation, has been used by Murphy and Pennock[23] to measure monosaccharides in blood and urine. Single peaks were obtained for glucose, fructose, mannose and xylose, but other monosaccharides gave two peaks, presumably due to formation of *syn*- and *anti*-isomers. One galactose peak coincided with that of glucose. A similar procedure has been used for disaccharide estimation by Schwind *et al.*[22], one peak being obtained for maltose, but two resulting from lactose. Allen *et al.*[24], measuring galactitol levels in liver and amniotic fluid, used the derivatisation procedure described by Murphy and Pennock[23] to modify the retention time of glucose and thus prevent it interfering.

Polyacetyl aldononitrile derivatives may be synthesised from aldoses by dissolving sugars in pyridine, adding hydroxylamine hydrochloride, and heating. Acetic anhydride is added, converting sugar oximes which have been formed to the corresponding aldononitriles, and acetylating free hydroxyl groups[25,26]. Polyols do not react with hydroxylamine hydrochloride, and thus form fully acetylated derivatives. This type of procedure has been used by Szafranek *et al.*[27] to separate 32 aldoses and polyols on glass capillary columns. The same group later separated and quantified 17 sugars and polyols occurring in human urine[28], and adapted the technique to the analysis of lens tissue from cataractous subjects[29]. A method for measuring D-mannose in serum as its aldononitrile acetate has been described[30], and this type of derivatisation has also been used to estimate sugar and sugar alcohol concentrations in bacteriological culture media[31].

Samples derivatised by the various acetylation procedures described have been

prepared for injection in several ways. Some authors have injected the reaction products directly onto the chromatography column[16,20,27], while others have first evaporated the reaction mixture and then dissolved the residue in a small volume of solvent, *e.g.*, chloroform[17,23], trichloromethane[30] or acetone[31], prior to injection.

2.2.2. Trifluoroacetyl derivatives. Derivatisation of sugars with trifluoroacetic anhydride to form the corresponding esters was originally described by Bourne *et al.*[32]. Vilkas *et al.*[33] resolved monosaccharides, disaccharides and trisaccharides as their trifluoroacetates, and showed that for glucose this derivative was more volatile than the corresponding trimethylsilyl ether. Trifluoroacetyl derivatives have been used for separating cyclical sugar alcohols[34], combined with alditol formation to measure sugars in plasma[35] and combined with methyloxime synthesis for disaccharide and trisaccharide analysis[22].

Several different trifluoroacetylation procedures have been described. Vilkas *et al.*[33] prepared derivatives by using trifluoroacetic anhydride and sodium trifluoroacetate in acetonitrile. Trifluoroacetic anhydride has also been used with pyridine[34], tetrahydrofuran[36] and dichloromethane[37] as solvents. More reproducible derivatisations have been claimed if N-methyl-bis(trifluoroacetamide) is used, with an equal volume of pyridine as solvent[38].

2.3. Trimethylsilyl derivatives

Trimethylsilyl (TMS) derivatives have been widely used for the analysis of sugar mixtures since Sweeley *et al.*[39] described a simple method of preparation. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and anhydrous pyridine was added to dried sugars, derivatisation occurring rapidly at room temperature with virtually quantitative yields. Good separations of various pentoses and hexoses were obtained although some isomeric polyols (particularly galactitol, mannitol and sorbitol) were not resolved. A fine precipitate of ammonium chloride was formed in the reaction mixture, and since this was injected directly, some contamination of the chromatographic column occurred.

This procedure has been adapted to the measurement of monosaccharides and sugar alcohols in blood, urine and tissues by Wells and co-workers[40–42], peaks being quantified by direct comparison with the appropriate standard. A similar derivatisation procedure has been used by Bhatti and Clamp[43] who modified the technique to include internal standardisation and temperature programming, measuring simultaneously urinary mono- and disaccharides. Ammonium chloride contamination of the column was avoided by De Neef[44] who extracted the products of derivatisation with hexane before injection. Sweeley-type derivatisations have been applied, with some modification, to the measurement of sugar alcohols in tissues[45–48], blood, cerebrospinal fluid and seminal fluid[40] and urine[50], and to the estimation of sugars in cerebrospinal fluid[51].

The TMS ethers of mannitol, sorbitol and galactitol, and those of isomeric pentitols are not as well resolved as the corresponding acetyl derivatives. Some authors report results as total hexitols and pentitols[52], while others include additional methods to differentiate individual sugar alcohols. These additional techniques include enzymatic and microbiological procedures[53], and GLC methods which include

acetylation in addition to trimethylsilylation[54]. Partial separation of mannitol from sorbitol and galactitol has been reported by using 2.7-m columns with a high percentage, moderately selective stationary phase (10% OV-17; ref. 55). TMS-hexitols and also TMS-pentitols have been resolved with capillary columns[2].

TMS ethers are susceptible to hydrolysis if traces of water or acid are present. This possibility may be reduced if more powerful silyl donors than HMDS are used, such as N,O-bis(trimethylsilyl)acetamide (BSA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), N-trimethylsilylimidazole (TMSI), or N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)[56,57]. TMSI has been used to derivatise sugars in urine[58], milk[59], erythrocytes[60] and cerebrospinal fluid[61]. Procedures using BSA, with TMCS as catalyst, have been described in which sugar alcohols, monosaccharides and disaccharides in plasma, urine and bile have been determined[55,62,63]. Although in theory BSTFA should be a self-catalyzing silyl donor, it has been found necessary to add TMCS as an additional catalyst for rapid quantitative derivatisation[56,62]. This combination of reagents has been used in methods to measure monosaccharides and polyols in blood and urine[64,65] and in the study of oligosaccharides in milk[66].

Pyridine is the solvent used for the majority of studies involving silylation although other solvents, such as dimethylformamide and dimethylsulphoxide, have also been used[67–70]. Among the advantages cited for dimethylformamide compared to pyridine are:

(1) when used with 2-hydroxypyridine as a catalyst, a more reproducible molar response factor is obtained, particularly for N-acetyl sugars;

(2) it is more pleasant to work with;

(3) it is more volatile, and causes less tailing of the solvent front.

Additional derivatisation procedures have been combined with trimethylsilylation to decrease the incidence of multiple derivatives. Combined alditol–TMS derivatives have not been used, since these compounds are less satisfactorily resolved than the corresponding acetyl esters. Sweeley *et al.*[39] described methods for the preparation of oxime–TMS sugars, and this procedure has been adapted to measure glucose in urine[52]. Since the retention times of monosaccharides are modified, additional separations may be achieved by this method, *e.g.* β-galactose and galactitol could not be resolved as TMS ethers, but were separated when the oxime–TMS derivative of galactose was formed[46]. The synthesis of methyloxime–TMS sugars has been applied to the quantitation of monosaccharides in plasma and urine[64,65] and seminal fluid[2]. The formation of oxime and methyloxime derivatives prior to silylation reduces the number of multiple derivatives. However, two peaks may result from a single sugar[56,71] presumably due to the formation of *syn*- and *anti*-isomers. Lactone and methylglycoside formation have been combined with trimethylsilylation but result in little reduction in the number of derivatives[4,5,39].

2.4. n-Butylboronate derivatives

Butylboronate derivatives have been shown to give useful separations of mannitol, sorbitol and galactitol[72], the derivatives being stable in the presence of water. As with other derivatives, multiple peaks were obtained from aldoses. One of those from glucose had the same retention time as mannitol. This derivatisation has been used as the basis for the quantitation of sugar alcohols in pharmaceutical

preparations[73] and has recently been adapted to the analysis of mannitol in urine[74]. In this method interference due to glucose was overcome by first reducing it to sorbitol.

2.5. Comparison of derivatives

Of the derivatives described, acetyl esters and TMS ethers have been most widely used for measuring sugar and sugar alcohol concentrations. The major advantages for acetyl esters are greater stability to water, and better resolution of isomeric polyols. TMS ethers are more volatile, a property of great advantage in the estimation of di- and trisaccharides. Methylation techniques have not been as widely used for measuring free sugar and polyol concentrations, probably because early methods of preparation were cumbersome. Methyl derivatives are more stable to water than TMS ethers and more volatile than acetyl esters. Trifluoroacetates are detectable at lower concentrations using an electron-capture detector than derivatives which are quantified with a flame-ionisation detector. Using trifluoroacetylation, Pritchard and Niedermeier[75] were able to quantitate the carbohydrate composition of 0.1 μg glycoprotein. Disadvantages of the technique are that the derivatives are extremely susceptible to hydrolysis, and some authors have reported unreliable quantitative results[76]. The principle advantage for *n*-butylboronate derivatives is that they resist hydrolysis by water.

3. COLUMNS AND STATIONARY PHASES

Stationary phases that have been used are presented in Table 1. Non-polar liquid phases have generally been used for separating TMS derivatives while acetyl esters have been resolved using more polar phases. The ratios of liquid to inert support have varied from 1 to 25% (w/w) although in general low percentages have been used. Gas-Chrom P, Gas-Chrom Q and Chromosorb W have all proved satisfactory as inert supports.

4. QUALITATIVE AND QUANTITAITVE ANALYSIS

4.1. Qualitative analysis

Identification and characterisation of sugars and sugar alcohols by GLC is dependent on several factors, *e.g.* the ability to separate different derivatives, the stationary phase used, the carrier gas flow-rate, and the column temperature. The latter two are difficult to reproduce precisely, but the effect of variations may be minimised by defining the position of peaks relative to marker substances. This is done by determining relative retention times or methylene unit values. The relative retention time is the ratio of the time for the test sugar to appear relative to that for a reference internal standard compound. Fluctuations in column temperature and carrier gas flow-rate would affect the test and internal standard retention times in proportion, and the relative retention time would not alter. Thus identification of sugars relative to the position of a marker peak is subject to less error than being dependent on an absolute retention time. Extensive lists of relative retention times

using several stationary phases have been published for different deivatives of monosaccharides, disaccharides and sugar alcohols[80,81], for TMS-carbohydrates[39], for TMS-disaccharides[62], for alditol acetates[16,17] and for oxime– and methyloxime–TMS-monosaccharides[56].

Characterisation by methylene unit values involves determining the position of each peak relative to two straight chain hydrocarbon standards, one with a shorter and the other with a longer retention time than the test peak, *e.g.* a sugar with a methylene unit value of 18.5 would appear half way between octadecane and nonadecane. This method has been used to characterise separations of methyl ethers of disaccharides[12], aldononitrile acetates of monosaccharides and polyols[27–29], and TMS derivatives of mono-, di- and trisaccharides[58].

Both methods use the time of the maximum peak height. Whether useful separations of two sugars with similar retention times will be achieved depends not only on the retention time but also on the width of the peaks (*i.e.* on the efficiency of the column). The column efficiency may vary considerably with different batches of stationary phase.

4.2. Quantitation

Quantitative results are usually obtained by using internal standardisation. A known amount of internal standard is added to the test samples and standard solutions at the start of the analytical procedure. The detector response ratio or molar response factor for each sugar of interest is determined relative to that of the internal standard. This must be calculated for each sugar since response factors cannot be predicted, because although the response should be proportional to the mass of the substance, detectors do not "see" all chemical groups equally well. This applies particularly to TMS derivatives[82]. Using the response factors obtained from standard solutions, the concentrations of sugars in test samples may be determined by measuring the ratio of test to internal standard.

Using an internal standard has three main advantages: (1) identification of test peaks is more certain; (2) samples are injected with a microlitre syringe with which it is difficult to deliver a precise volume. With internal standardisation a ratio is measured and thus fluctuations in volume may be compensated for, and (3) volatile solvents may be used and some evaporation of these may occur. With internal standardisation these losses are corrected.

An "ideal" internal standard should have certain properties: (a) it should be chemically similar to the substances being estimated; (b) it should give a single chromatographic peak which is well separated from other components; (c) it should be stable; (d) it should be available in a highly purified form and (e) it should not occur naturally in the fluid being analysed.

Two groups of substances have been used as internal standards for sugar analyses, other sugars and hydrocarbons. Both give reference peaks for establishing retention data and both will compensate for variations in injection volume. An advantage of using a sugar or polyol is that since the internal standard will also be derivatised any variables affecting this procedure should affect test and standard substances equally. Also, test and standard sugars should be equally affected by molecular sieving or water displacement effects that may occur if an ion-exchange

TABLE 1

STATIONARY PHASES USED FOR SUGAR AND SUGAR ALCOHOL ANALYSIS

Stationary phase	*Derivative*	*Application*	*Ref.*
Methyl silicone			
1% SE-30	Methyl	Disaccharides in faeces	12
3% SE-30	TMS	Monosaccharides in cerebrospinal fluid	51
		Myoinositol in biological fluids	49, 50
		Galactitol in biological fluids	77–79
		Monosaccharides in erythrocytes	60
	Oxime-TMS	Glucose and polyols in urine and tissues	52
3.8% SE-30	TMS	Mono- and disaccharides in urine	43
5% SE-30	TMS	Sugars in urine	58, 64
2% OV-1	TMS	Sugars in biological fluids	67
3% OV-1	TMS	Disaccharides in blood and urine	62
12% OV-1	TMS	Monosaccharides and sugar alcohols in blood	65
Phenylmethyl silicone			
3% SE-52	TMS, acetyl	Serum monosaccharides and polyols	54
	TMS	Polyols in urine	53
		Inositol in tissues	45
1% OV-17	Methyl	Disaccharides in faeces	12
2% OV-17	TMS	Carbohydrates in biological fluids	67
3% OV-17	Acetyl	Mannitol in urine	55
5% OV-17	Butylboronate	Mannitol in biological fluids	74
10% OV-17	TMS	Sugars and polyols in biological fluids	55, 62, 63
Trifluoropropyl methyl silicone			
3% QF-1	TMS	Urinary disaccharides	62
5% QF-1	Aldononitrile, acetyl	Monosaccharides and polyols in culture media	31

Cyanoethyl methyl silicone			
3% XE-60	TMS	Disaccharides in urine	62
		Inositol in tissues	47
2% XF-1105	Alditol formation + trifluoroacetylation	Sugars in blood	35
Cyanopropyl methyl silicone			
3% SP 2340	Acetyl	Galactitol in amniotic fluid	24
Phenylcyanopropyl methyl silicone			
1 and 5% OV-225	Acetyl	Polyols in cerebrospinal fluid and plasma	20
Vinylmethyl silicone			
3.8% CCW-98	TMS	Sugars and polyols in biological materials	44
Ethylene succinate silicone copolymers			
3% ECNSS-M	Methoximeacetyl	Monosaccharides in blood and urine	23
	Acetyl	Polyols in blood and urine	18
1% EGSS-X	TMS	Polyols in tissues	47
Ethylene glycol succinate			
15% EGS	TMS	Polyols in biological fluids	45, 47, 53
		Sugars in serum and urine	40
25% DEGS	TMS	Inositol in tissues	48
Polyamide			
3% poly 101A	Acetyl	Polyols in serum and urine	54
Polypropylene glycol			
5% UCON LB-550	TMS	Inositol in tissues	48
Capillary columns			
SE-30	Aldononitrile acetyl	Monosaccharides and polyols in urine and the lens	27–29
	Methoxime-TMS	Monosaccharides and polyols in seminal fluid	2
SE-52	TMS	Polyols in cerebrospinal fluid	61

or protein precipitation stage is included. Hydrocarbons are chemically dissimilar but have the advantage that they do not occur naturally in biological fluids, and from the great number available one may be easily selected with a retention time which falls within a vacant portion of the chromatogram.

The following hydrocarbons and related substances have been used: glyceryl-1-decyl ether[27-29], *n*-dodecanol[61], diphenylbenzene[74] and diethylphthalate ester[83]. Sugars and polyols that have been used include α-methylglucose[55,62], α-methylmannose[67], 3-O-methylglucose[35], mannitol[23,43,81], L-rhamnose[44], deoxyribose[65], trehalose[35], turanose[62], cellobiose[35], sedoheptulosan hydrate[53], perseitol[31] and α-methylxylose[54]. Other substances that have been used include uridine[43]. Several of these standards do not conform with the requirements listed above. Anomers of 3-O-methylglucose, L-rhamnose, cellobiose and deoxyribose will occur unless double derivatisation procedures are employed. Mannitol occurs naturally in urine and is not suitable as an internal standard when urinary sugars are being determined[63].

Some authors have not used internal standardisation but have relied on careful attention to volumetric technique[20,30,40,45,49]. However, when the two methods of quantitation have been compared greater precision has been achieved with internal standardisation[62,84].

Peak height or area measurement may form the basis of quantitation if isothermal analyses are being performed, but it is customary to measure peak area when temperature programming is used. Partially overlapping peaks may be more satisfactorily quantified by measuring peak height[62].

Sugars that anomerise may be quantified in different ways. It is sometimes possible to select a stationary phase on which the anomers coincide. Some authors have measured the area of each anomer and added them to give a total peak area for that sugar[81], or measured a combined peak height[67]. Others have reported satisfactory results by using a single anomer for quantitation[62].

4.3. Detailed analytical procedure

The stages included in a typical analytical procedure are shown in Fig. 1.

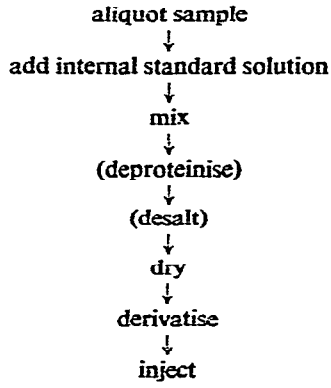

Fig. 1. Scheme for analytical procedure. The stages in parentheses are not always required.

Deproteinisation of serum samples is necessary to prevent excessive consumption of derivatisation reagents which would result in dilution of the sugars with

consequent loss of sensitivity. A variety of reagents have been used for protein precipitation, including the Somoygi technique[40,65], ethanol[44,62], perchloric acid[19] and sulphosalicylic acid[62]. Separation by ultrafiltration has been described in several studies[2,52,54]. With Somoygi and ethanol precipitation large volumes result and the fluid must be removed before samples can be derivatised.

In many methods additional purification of samples is undertaken. Urea in high concentrations has been reported to interfere with silylation reactions[85]. Some authors remove urea by incubating with urease[40,52], others by separating sugars from urea by column chromatography[50]. It has been shown that the mixed-bed resin Zerolit DM-F (BDH, Poole, Great Britain) reduces urea concentrations[86] and this has been used to treat samples for sugar estimation[55,62]. Procedures for urinary estimations have been reported which do not include urea removal[43,44].

Desalting procedures may be necessary to remove polar constituents which might interfere with derivatisation. Excess protein precipitants and ammonia generated from urea breakdown are usually removed by this technique. A mixed-bed resin[40,55,62] or anionic and cationic exchange resins[18,52] may be used. Samples are then dried by evaporation[19,55,62], lyophilisation[27,54] or desiccation[43], following which the residues are derivatised.

5. PERFORMANCE OF ASSAYS

As for any method, GLC may be assessed for accuracy (recovery), precision, sensitivity, linearity, interferences and comparison with other techniques.

Recoveries are usually determined by adding sugars to the fluid being investigated and estimating the amount present which is then quoted as a percentage of the amount added. These values are usually apparent rather than true recoveries since the amount determined is relative to that of the appropriate standard. When internal standardisation is used recoveries of 90–110% are obtained for acetylation and trimethylsilylation procedures. Monson and Wilkinson[30] obtained values of 85–113% for mannose determination in plasma using aldononitrile acetylation without internal standardisation. Recoveries of 94–104% for glucose determination were obtained by Wells *et al.*[40] who formed TMS ethers using a volumetric technique. With the same method of quantitation, Servo *et al.*[20] found somewhat lower values when polyols were measured as acetyl derivatives in cerebrospinal fluid (75–90%).

Gas chromatographic methods are relatively precise, particularly when internal standardisation is used. Intra-assay coefficients of variation of 2–6% have been obtained for several sugars and polyols in plasma and urine[55,62]. Using volumetric technique values of 2–12% have been reported[30].

One of the principle advantages of GLC is its great sensitivity. Several procedures have been described which will quantify concentrations of 1–10 mg/l (refs. 43, 46, 55 and 62).

Unrelated substances, in addition to other sugars, may have a similar retention time to the sugar being estimated. Specificity may be checked by analysing on several stationary phases and by using alternative derivatisation procedures, since it is unlikely that an interfering substance will co-chromatograph with several different methods. If necessary, the structural nature of a peak may be characterised by combined gas chromatography–mass spectrometry.

Glucose determinations by GLC have been compared with several glucose oxidase-based methods, correlation coefficients of 0.98 and 0.99 being obtained[67]. A similar correlation coefficient was found when lactulose estimations by quantitative paper chromatography and GLC were compared[71]. These results show that the accuracy of GLC methods for sugar determinations is comparable with these alternative techniques.

6. CONCLUSIONS

Many GLC procedures are available for measuring sugars and sugar alcohols in biological fluids. They should be considered where it is necessary to measure these substances simultaneously, to resolve complex mixtures, or to quantify low concentrations.

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8. SUMMARY

Gas–liquid chromatographic procedures for measuring sugar and polyol concentrations in biological fluids are reviewed. Such methods require the preparation of derivatives such as methyl ethers, trimethylsilyl ethers or acetyl esters.

Prior to derivatisation samples must be deproteinised and dried. Complex mixtures of sugars and sugar alcohols may be resolved. Quantitative analyses are precise, sensitive and linear. If internal standardisation is used recoveries approaching 100% are obtained.

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