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EVALUATION OF THE MASS DETECTOR FOR QUANTITATIVE DETECTION OF TRIGLYCERIDES AND FATTY ACID METHYL ESTERS

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SUMMARY

The mass detector, a light scattering detector for use with high-performance liquid chromatography systems, was studied in order to determine which factors might affect sensitivity and quantification in the determination of triglycerides and other lipid materials.

The detector response was found to be non-linear for triglycerides. The sensitivity was not only affected by evaporation temperatures and atomiser inlet air pressures within the detector, but also by the mobile phase flow-rate, and its composition.

Detection limits of less than 1 μg were achieved for non-volatile, saturated triglycerides, by direct injection, and the reproducibility of responses was about ± 6% with isocratic elution (excluding tributyrin which was too volatile to be quantified accurately), but somewhat greater (± 16%) with gradient elution.

INTRODUCTION

The analysis of triglycerides by high-performance liquid chromatography (HPLC) is becoming increasingly popular. Most workers use non-aqueous reversed-phase chromatography, which separates on the basis of equivalent carbon number[1–3], although argentation chromatography, where separation is on the basis of unsaturation[4,5], is sometimes used. The development of gradient elution systems has facilitated the separation of complex triglyceride mixtures, such as those encountered in butter[1,6–8], however, detection of lipids and particularly triglycerides still remains a problem.

There are many different types of detector available, and all of them have some limitations. There are currently five types of detector which have been used for the detection of triglycerides: infra-red (IR), transport flame ionisation (FID), refractive index, ultraviolet (UV), and light-scattering detection. Apart from refractive index detectors all of these are compatible with gradient elution although IR detection suffers from base-line drift problems[2] and UV detection is incompatible with one of the most suitable solvents (acetone)[1]. Transport flame ionisation detectors have not been commercially available for some time, although recently a new detector has

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been brought onto the market (Tracor, Model 945). The old detectors were particularly useful, in that the eluting solvent was removed from the analyte prior to detection and hence gradient elution was possible, however they were of low sensitivity[9,10]. Unfortunately, no experience with FID is available. The fifth type of detection system, light-scattering, can be used with gradient elution, using any volatile solvents because, just as is the case with FID, the solvent is evaporated before detection of the analyte. However, unlike FID all the eluate may be analysed and hence sensitivity is potentially greater.

A preliminary examination of the use of a commercially available light-scattering detector, the mass detector, for the detection of triglycerides has been made, by comparison with refractive index and UV detection[11]. The basis of the detection system in the mass detector is light scattering. The eluate from a chromatographic column is atomised with the aid of air under pressure, and as the fine mist of liquid droplets passes down the heated stack solvent is evaporated. At the bottom of the stack, if analyte is present a mist will remain in the form of either concentrated liquid droplets or particles, depending upon the analyte and conditions of temperature and pressure. These droplets or particles then pass through a beam of light, and the light that is scattered at an angle of 120° is detected and taken as a measure of the amount of material present[1,6,11].

Preliminary work had indicated that the mass detector might be useful for triglyceride analysis[11], however, little was known about its sensitivity and suitablility for quantitative analysis. The literature available on experimental light-scattering detectors[12,13] and laser light-scattering detectors[1,6] suggested that, in addition to instrumental factors, *i.e.* attenuation and photomultiplier sensitivity, atomisation air pressure and evaporation temperature might also affect sensitivity. It was therefore considered necessary to conduct a thorough study of these factors which might affect sensitivity and reproducibility of the detector response prior to using the mass detector for quantitive analysis of triglycerides and other lipid classes. The results of this study are presented below.

MATERIALS AND METHODS

Most of the work was carried out with a Gilson Model 704 gradient chromatograph with computerised integration and data handling (Anachem, Luton, U.K.). Although, in some instances (where stated below), a Bryans BS600 and a Hewlett-Packard 3390A integrator were used in conjunction with the Gilson chromatograph. A mass detector 750/14 (Applied Chromatography Systems, Luton, U.K.) was used. Detector settings were as follows (unless otherwise stated in the text): attenuation, × 1; photomultiplier, × 2; time constant, 5; evaporator, 30; air inlet pressure, 24 p.s.i. Injection was achieved through a Rheodyne valve 7125 (fitted with a 20-μl loop) either directly into the detector or onto two Spherisorb-5-ODS 2 (Phase Separations, Gwent, U.K.) columns in series (50 × 4.6 mm and 250 × 4.6 mm) which had been packed from acetone in our own laboratories. In all cases 5-μl injections were made in triplicate. All solvents were of analytical reagent grade (Fisons, Loughborough, U.K.) except for acetonitrile which was of HPLC grade (Rathburn Chemicals, Walkerburn, U.K.). Triglycerides and fatty acid methyl esters (FAMEs) were all of high purity: *ca.* 99% (Sigma, Poole, U.K.).

Sensitivity

Detection limits were determined by direct injection and seven series of dilute solutions (0.02–0.2% or 0.4%, w/v) of triglycerides in acetone were prepared. The triglycerides studied were: tributyrin, trihexanoin (tricaproin), trioctanoin (tricaprylin), tridecanoin (tricaprin), trilaurin, trimyristin and tripalmitin. Direct injections were made, as described above, and peak height measurements were taken from the chart produced. Elution was with acetone at a rate of 1 ml min^{-1}.

In addition, detection limits were determined for the same triglycerides after isocratic elution from a chromatographic column under the following conditions: Spherisorb-5-ODS 2 column (250 × 4.6 mm), acetone–acetonitrile (65:35, v/v), at a flow-rate of 1 ml min^{-1}; detector settings: attenuation, × 2; photomultiplier sensitivity, × 2; time constant, 5; evaporator, 50; air inlet pressure, 22 p.s.i.

Linearity

The linearity of response in the range 3–150 μg was investigated for six FAMEs: methyllaurate, methylmyristate, methylpalmitate, methylstearate, methyloleate and methyllinoleate (dissolved in acetone). Injection of each individual FAME was made onto a column and elution was with acetone, 2 ml min^{-1}. Evaluation was through peak areas determined using the Hewlett-Packard integrator. The linearity of response was also investigated for saturated and unsaturated triglycerides by gradient elution. Two series of triglycerides were made using tetrahydrofuran as a solvent. One series contained the saturated triglycerides tributyrin, tricaproin, tricaprylin, tricaprin, trilaurin, trimyristin and tripalmitin. The other series contained the unsaturated triglycerides tripalmitolein, triolein, trilinolein and trilinolenin. Non-linear gradient elution at a flow-rate of 2 ml min^{-1} was used for the saturated and unsaturated triglycerides as follows:

Saturated		*Unsaturated*	
Time (min)	*Acetone in acetonitrile (%)*	*Time (min)*	*Acetone in acetonitrile (%)*
0	15	0	75
2.33	20	5	75
6.37	95	12.5	87.5
20	100	14.5	75
25	100	25	75
28	15	Run time 15 min plus 10 min wash cycle	
45	15		

Run time 25 min plus 20 min wash cycle

All other conditions are as described above except that the air inlet pressure on the detector was 22 p.s.i.

Reproducibility

Multiple injections were made for one sample from each of the unsaturated and saturated triglyceride series above with both gradient and isocratic elution. Reproducibility of response was determined from these data.

Effect of evaporator temperature setting on detector responses

A series of solutions of methyl palmitate in acetone was prepared. Direct injections were made at each of the following evaporator settings: 24, 26, 28, 30, 32, 34. (It should be noted that these settings do not correspond to any absolute measurement of temperature at any particular part of the mass detector. However, the higher the number the greater the overall temperatures.) Elution was with acetone, 1 ml min^{-1}, and the air inlet pressure was 22 p.s.i.

One of the series of saturated triglyceride solutions described in the Linearity section was used to determine the effect of evaporator setting on the detector response to triglycerides at evaporator settings of 24, 26, 28, 30, 35 and 40; the triglycerides being eluted from a column, using a solvent gradient as described above (Linearity section).

Effect of atomiser inlet air pressure on detector response

One of the series of saturated trigliceride solutions described in the Linearity section was used to determine the effect of inlet air pressure on the detector response to triglycerides at inlet air pressures of 22, 24, 26 and 30 p.s.i.

Effect of solvent flow-rate on detector response

Tricaprin (2.81 mg ml^{-1}) and trilaurin (2.65 mg ml^{-1}) solutions were prepared in acetone. Direct injections were made at solvent flow-rates of 0.5, 1.0, 1.5, 1.75, 2.0 and 3.0 ml min^{-1} with acetone as the eluting solvent. Evaluation was from peak areas determined using the Hewlett-Packard integrator.

Solvent effects on detector response

Trilaurin and tricaprin were used to investigate the effects of solvent on the detector response to triglycerides. A series of solutions (0.04–1.2%, w/v) of each triglyceride was prepared in each of the following solvents: acetone, acetonitrile, 2-propanol and chloroform. Direct injections were made, for each series, using the same solvent for elution as was used to dissolve the sample. The flow-rate used was 1 ml min^{-1} and integration was with the Hewlett-Packard instrument. Peak areas were interpolated to correspond to an injection of 25 μg in 5 μl (*i.e.* 0.5%, w/v).

Example of application

Butter fat contains a complex mixture of triglicerides and its separation by non-aqueous reversed-phase HPLC benefits from the use of gradient elution. An acetone solution of unsalted english butter was chromatographed under the following conditions: Spherisorb-ODS-2 5-μm column (250 × 4.6 mm I.D.) with a 50 × 4.6 mm I.D. guard column. Elution was with acetone in acetonitrile, by linear gradient from 20% acetone to 90% acetone, at a flow-rate of 1.5 ml min^{-1}. Mass detector settings were: attenuation, × 1; photomultiplier sensitivity, × 2; time constant, 5; evaporator setting, 25; air inlet pressure, 22 p.s.i.

RESULTS AND DISCUSSION

Detection limits

The detection limits for seven triglycerides were determined by direct injection

of triglyceride solutions. A log–log plot of peak area against weight of triglyceride injected gave an almost linear plot (see following section), hence the weight of triglycerides required to give a peak height of three times the height of the base-line noise was calculated from the regression for each triglyceride. The detection limits are presented in Table I.

TABLE I

MASS DETECTOR DETECTION LIMITS FOR SATURATED TRIGLYCERIDES AS DETERMINED BY DIRECT INJECTION AND AFTER COLUMN CHROMATOGRAPHY

Triglyceride	*Detection limits*	
	*Direct injection** (μg/5 μl)*	*After column** chromatography (μg/5 μl)*
Tributyrin	3.14	8.41
Tricaproin	0.99	1.41
Tricaprylin	0.97	0.69
Tricaprin	0.64	0.71
Trilaurin	0.92	0.92
Trimyristin	0.33	1.45
Tripalmitin	3.03	3.67

* Acetone, 1 ml min^{-1}; attenuation, × 1; photomultiplier, × 2; evaporator, 30; inlet air, 24 p.s.i. No column.

** Spherisorb-5-ODS 2 (250 × 4.6 mm); acetone–acetonitrile (65:35, v/v); flow-rate 1 ml min^{-1}; attenuation, × 2; photomultiplier, × 2; evaporator, 50; inlet air, 22 p.s.i. This detector was a Mark 1 mass detector.

The detection limits are less than 1 μg for all the triglycerides except the two extreme cases: tributyrin and tripalmitin. The tributyrin has a relatively low boiling point (190°C)[14] and it is probable that the reduced sensitivity is due to vaporisation. In the case of the tripalmitin, however, solubility is a more probable cause of the reduced sensitivity. Triplamitin has only a limited solubility in acetone and will readily precipitate which would tend to encourage losses prior to nebulisation. If this were not the case a response similar to the other less-volatile triglycerides would be expected, *i.e.* a relatively high response, because tripalmitin would be present as particles at the point of detection and hence should give a greater response than more volatile materials which are present as liquids at the point of detection[6]. After injections of tripalmitin had been made the injection valve was washed by repeated injection of solvent, which produced peaks of decreasing size on the chart recorder strongly suggesting that tripalmitin had precipitated[15].

These detection limits are somewhat higher than those quoted for an experimental light-scattering detector, which uses a laser light source[1], however, they are adequate for the detection of triglycerides which are generally difficult to detect[11]. Detection limits for sugars are between about 0.5 and 1 μg for the mass detector[16]. These detection limits are in the same order as, or slightly better than those quoted for refractive index detection (1–5 μg)[17].

The detection limits obtained after chromatography (Table I) are similar, but generally higher than, those obtained by direct injection. In both cases (if the volatile tributyrin is ignored) there is a tendency for reduced sensitivity as molecular weight increases. This phenomenon has also been observed for refractive index detection[15]. In the case of refractive index detection band broadening of the later eluting higher-molecular-weight triglycerides was implicated and to some extent the detection limits observed after chromatography are in agreement with this hypothesis. However, in the case of tripalmitin the direct injection data indicate that losses of tripalmitin due to poor solubility are mainly responsible for the reduced sensitivity to this particular triglyceride.

Linearity of response

Fatty acid methyl esters (FAMEs). The linearity of the detector was investigated for six FAMEs by injecting onto a column. The response for each FAME was linear over the range studied (Fig. 1). However, the slope varied markedly with chain length and unsaturation. The response was decreased more by a decrease in the number of carbon atoms than by an increase in the number of double bonds, the less volatile FAMEs having higher responses at any given concentration. This effect of decreased response with decreasing molecular weight appears to be much more marked for the mass detector than the light-scattering detector[6], under the conditions used.

Clearly, from this data the mass detector is not mass responsive for FAMEs as had been suggested[12]. However, as they are volatile and the differences in vapour

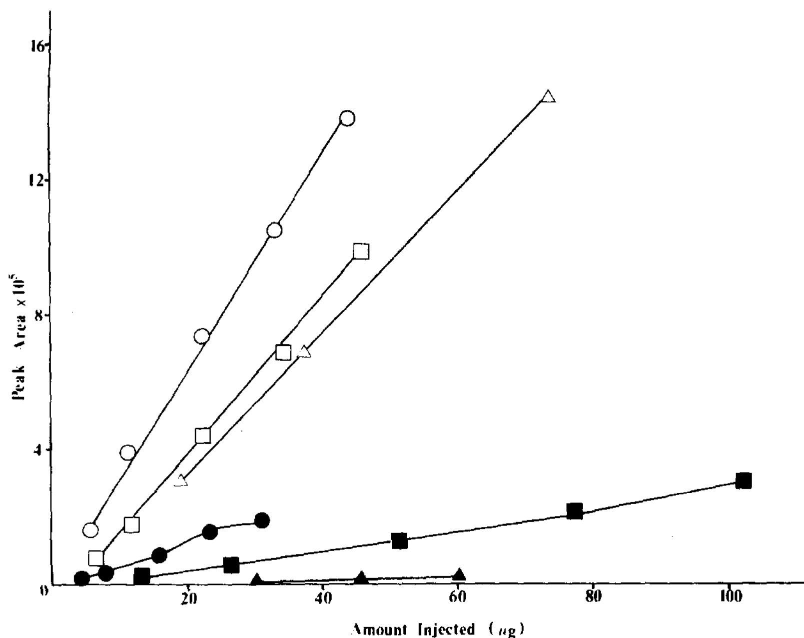

Fig. 1. Linearity of the response of the mass detector to six fatty acid methyl esters: methylstearate, ○; methyloleate, □; methyllinoleate, △; methylpalmitate, ●; methylmyristate, ■; methyllaurate, ▲. Hewlett-Packard integrator.

pressure between them and solvent are relatively small, the absence of a mass response can be attributed to vaporisation in this case. It might be expected therefore, to observe a mass response to the high-molecular-weight triglycerides but not to the lower-molecular-weight triglycerides if the mass detector is truly mass responsive.

Triglycerides. The linearity of the detector was investigated for seven saturated and four unsaturated, homogenous triglycerides. The response appeared to be linear in the range 10–25 μg on column for the saturated triglycerides (Fig. 2) and linear in the range 10–60 μg on column for the unsaturated triglycerides (Fig. 3). Here, as previously noted for FAMEs, the mass detector was not mass responsive, although some groups giving similar responses can be seen: (a) tricaproin, tricaprylin and tricaprin; (b) triolein and trilinolein; and (c) tripalmitolein and trilinolenin. The limited range of investigation for the saturated triglycerides is due to their poor solubility characteristics. The response curves were sigmoidal as might be expected from previous work on sugars using the mass detector[16].

Stolyhwo *et al.*[6] had shown that the response of their laser light-scattering detector, although not linear over the range that they examined, gave a straight line logarithmic plot. In addition, they demonstrated that the slopes of these straight line plots (log area *versus* log sample amount) were similar for all the solutes that they had examined, and they suggested that this indicated a similar mode of response for all the solutes. Logarithmic plots were made for the saturated (Fig. 4) and the unsaturated (Fig. 5) triglycerides, and the regression data are presented below in Table

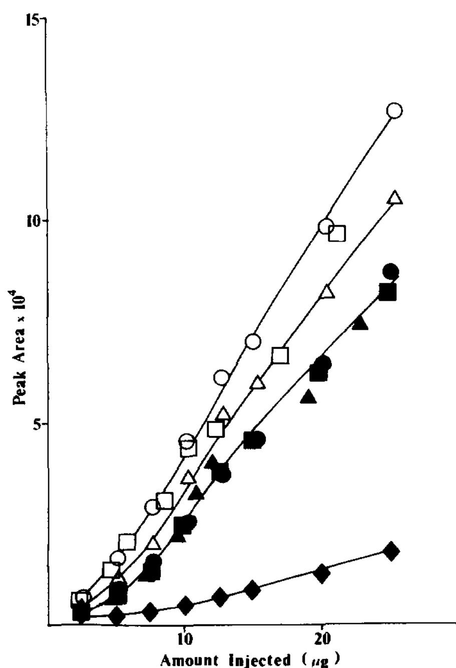

Fig. 2. Linearity of the response of the mass detector to seven saturated triglycerides: tripalmitin, □; trimyristin, ○; trilaurin, △; tricaproin, ●; tricaprylin, ▲; tricaprin, ■; tributyrin, ◆. Data Master integration.

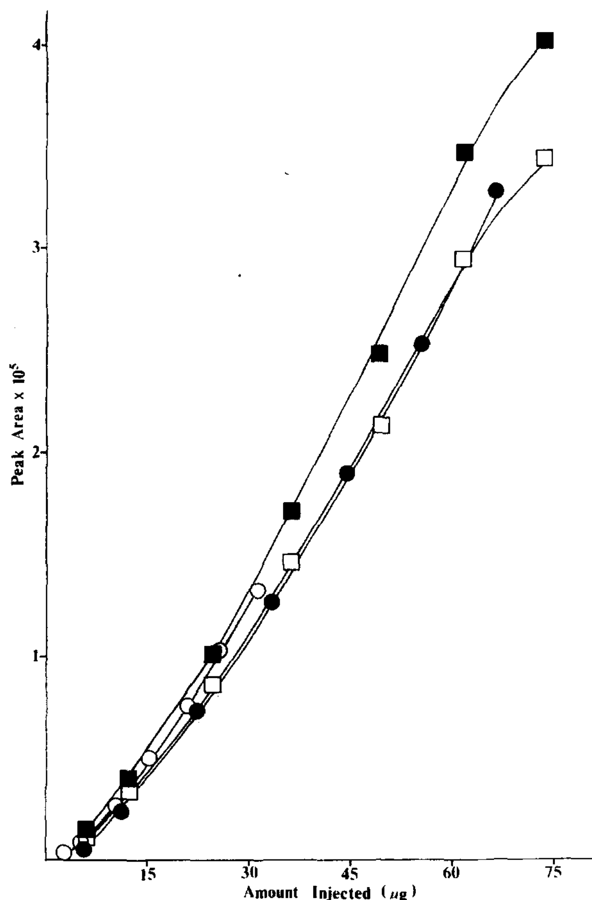

Fig. 3. Linearity of the response of the mass detector to four unsaturated triglycerides: tripalmitolein, ■; trilinolenin, ○; triolein, ●; trilinolein, □. Data-Master integration.

II. Unlike the laser light-scattering detector the slopes vary from one triglyceride to the next, implying that the mode of response may differ in each case.

The data indicate that the mass detector is not mass responsive for either of the lipid classes examined and that calibration is therefore necessary for all materials. This is undesirable from the point of view of quantitative analysis of triglycerides as a mass responsive detector would have proved invaluable for the quantitation of the mixed triglycerides which are expensive to purchase (when available) and difficult to prepare.

Reproducibility

The data for reproducibility are presented in Tables III and IV for both isocratic and gradient elution conditions. Under conditions of isocratic elution (Table III), the reproducibility is acceptable with coefficients of variation of less than 6% for all triglycerides except tributyrin. The tributyrin is very volatile and also gives a very low response per unit mass, factors which both contribute to the poor repro-

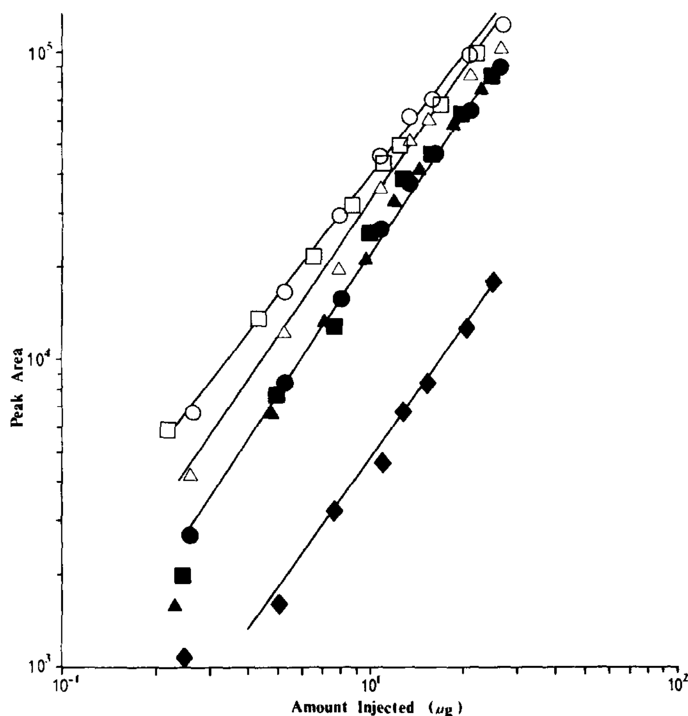

Fig. 4. Log/log plot of mass detector response against amount of triglyceride injected for seven saturated triglycerides: tripalmitin, □; trimyristin, ○; trilaurin, △; tricaprin, ■; tricaprylin, ▲; tricaproin, ●; tributyrin, ◆. Data Master integration.

TABLE II

REGRESSION DATA FOR LOGARITHMIC PLOTS OF DETECTOR RESPONSE AGAINST AMOUNT OF TRIGLYCERIDE

Triglyceride	*Number of observations*	*Correlation coefficient*	*Slope*	*Intercept*
Tributyrin	8	0.989	1.272	2.42
Tricaproin	8	0.998	1.519	2.83
Tricaprylin	8	0.994	1.663	2.67
Tricaprin	8	0.994	1.629	2.73
Trilaurin	8	0.996	1.420	3.06
Trimyristin	8	0.998	1.280	3.31
Tripalmitin	8	0.998	1.190	3.38
Tripalmitolein	7	0.999	1.35	7.16
Triolein	7	0.997	1.56	6.26
Trilinolein	7	0.999	1.41	2.95
Trilinolenin	7	0.999	1.54	2.85

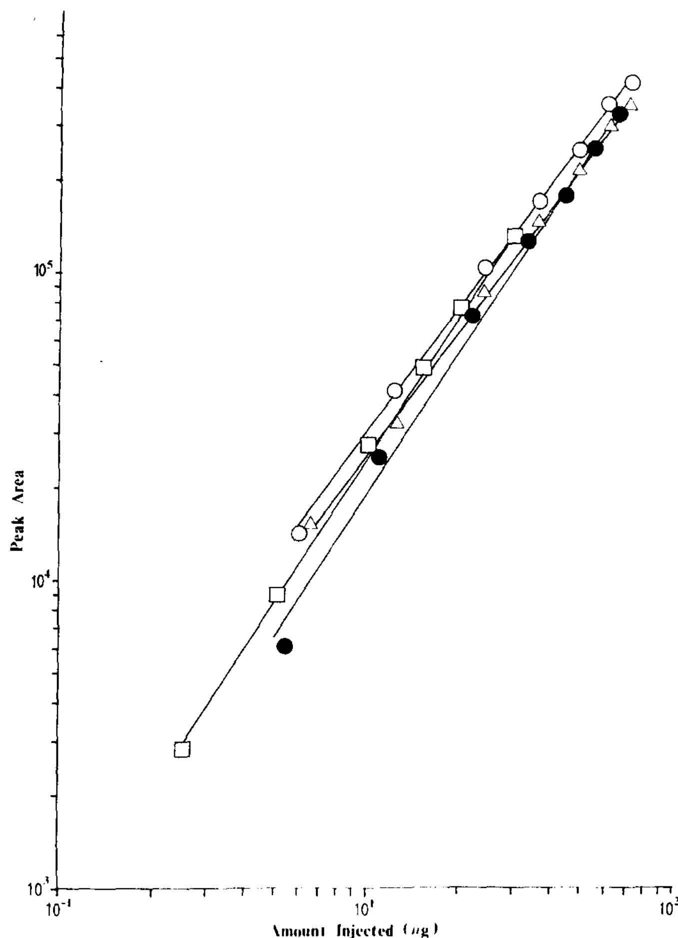

Fig. 5. Log/log plot of mass detector response against amount of triglyceride injected for four unsaturated triglycerides: tripalmitolein, ○; trilinolein, △; trilinolenin, □; triolein, ●. Data Master integration.

ducibility. These figures are slightly better than those quoted for refractive index detection with isocratic elution for saturated and partly unsaturated triglycerides, the coefficient of variation being 8.1% and 10.6%, respectively[18].

Under conditions of gradient elution (Table IV) the reproducibility is just acceptable for the unsaturated triglycerides (coefficient of variation in the order of 6.5%) but very poor in the case of the saturated triglycerides. The gradient used for the unsaturated triglycerides was very shallow, whereas that for the saturated triglycerides was particularly steep and it seems possible that the changes in solvent composition may be responsible for the poorer reproducibility of response observed with gradient elution. Hence, the effect of eluting solvent composition on response was investigated further (see Results and discussion, Solvent effect on detector response).

TABLE III

REPRODUCIBILITY OF RESPONSE FOR TRIGLYCERIDES ANALYSED USING A MASS DETECTOR UNDER CONDITIONS OF ISOCRATIC ELUTION

Triglyceride	*Number of injections*	*Mean peak area*	*Standard deviation*	*Coefficient of variation*
Tributyrin	9	5685	962	16.92
Tricaproin	9	387724	17297	4.46
Tricaprylin	9	582653	14132	2.43
Tricaprin	9	607214	31339	5.16
Trilaurin	9	578715	25970	4.49
Trimyristin	9	468651	21902	4.67
Tripalmitin	9	238942	13317	5.57
Tripalmitolein	8	477052	25126	5.27
Triolein	8	287653	15118	5.26
Trilinolein	8	398518	23314	5.85
Trilinolenin	8	124273	5459	4.39

TABLE IV

REPRODUCIBILITY OF RESPONSE FOR TRIGLYCERIDES ANALYSED USING A MASS DETECTOR UNDER CONDITIONS OF GRADIENT ELUTION

Number of injections 8.

Triglyceride	*Mean peak area*	*Standard deviation*	*Coefficient of variation*
Tributyrin	4101	654	15.95
Tricaproin	138658	12254	8.84
Tricaprylin	152202	10349	6.80
Tricaprin	140889	10763	7.64
Trilaurin	192277	30197	15.70
Trimyristin	259945	31240	12.02
Tripalmitin	243521	8434	3.46
Tripalmitolein	410249	27882	6.80
Triolein	326815	20928	6.40
Trilinolein	349120	20955	6.00
Trilinolenin	131207	8339	6.36

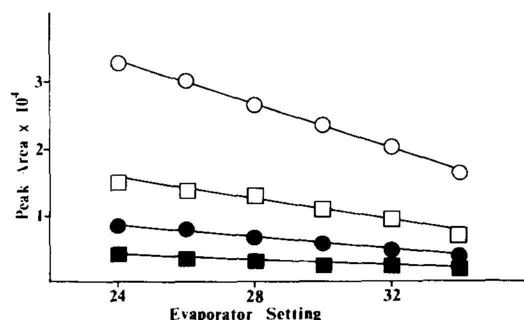

Fig. 6. Effect of detector temperature on response to methylpalmitate. Evaporator settings do not correspond to absolute temperature measurements, however, higher numerical values indicate higher temperatures. ■, 3.02 μg; ●, 5.81 μg; □, 11.16 μg; ○, 21.47 μg. Hewlett-Packard integration.

Effect of evaporator temperature setting on detector response

Fatty acid methyl esters. The influence of evaporator temperature setting on the response of the mass detector to methyl palmitate is shown in Fig. 6. In this case the relationship is simple, an increase in evaporator temperature setting causing a decrease in detector response. As the boiling point of methyl palmitate is low, 136°C (760 mmHg)[19] the higher temperatures cause more evaporation of the methyl palmitate and hence the reduced responses.

Triglycerides. The influence of evaporator temperature setting on the response of the mass detector to seven saturated triglycerides is shown in Fig. 7. The detector response, at various temperatures follows a similar pattern for all of the triglycerides except the extreme cases of tributyrin, tripalmitin and to some extent trimyristin.

Tributyrin has a low boiling point and is relatively volatile, hence the decreased response at increased evaporation temperatures is simply due to volatilisation.

The temperature optimum for tricaproin, tricaprylin, tricaprin and trilaurin is 30 (evaporator setting). Stolyhwo *et al.*[6] have suggested that this type of change in response is indicative of a change in the light-scattering properties of the analyte. They suggest that when the evaporation temperature falls below the melting point of the analyte that the material will be particulate when it passes through the light beam, and furthermore that as light is more strongly scattered by crystals than by liquid droplets that the response would be greater. Certainly, the pattern of response for the four triglycerides, over a range of temperatures, does appear to be the same as that observed by Stolyhwo *et al.*[6] for methylstearate and methylarachidate, however one notable feature is absent. The sudden decreases in detector response to methylstearate and methylarachidate occur at different temperatures, corresponding

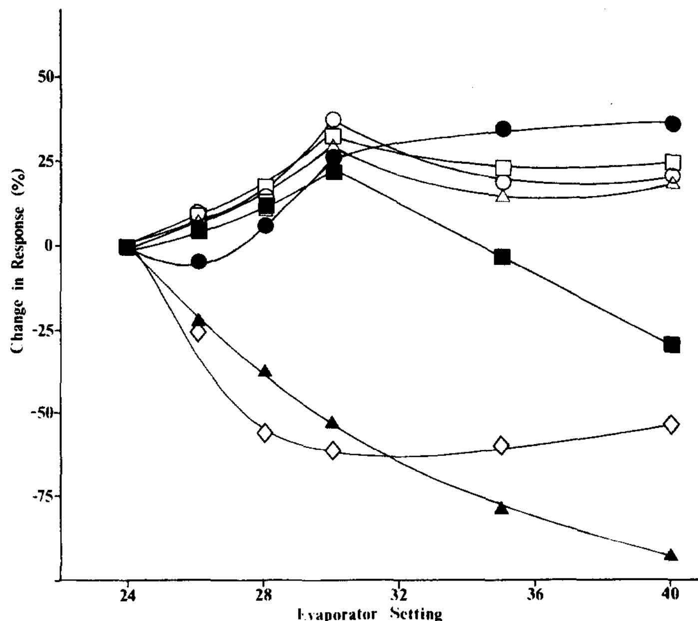

Fig. 7. Effect of detector temperature on response to seven saturated triglycerides: tripalmitin, ◇; trimyristin, ●; trilaurin, ○; tricaprin, □; tricaprylin, △; tricaproin, ■; tributyrin, ▲.

closely to the melting points of these esters. Nevertheless, although we have no accurate indication of the actual temperature at each evaporator setting it would be expected that the temperature optima for the triglycerides would be in ascending order (corresponding to their melting points). At present these observations have not been satisfactorilly explained.

The behaviour of methylmyristate and methylpalmitate does not follow the same pattern as the four triglycerides, however that of methylmyristate appears to be intermediate between that of methylpalmitate and the other triglycerides, implying a change in response mechanism.

These results indicate that good temperature control is essential in order to obtain reproducible, quantitative data.

Effect of atomiser inlet air pressure on detector response

The effect of four inlet air pressures on the response of the detector to the seven saturated triglycerides after chromatographic elution (as described in Materials and methods) was studied and the results are presented in Fig. 8. In all cases an increase in inlet air pressure caused a decrease in detector response, and this is in agreement with data for a similar detector[6]. These results indicate that careful control of the atomiser inlet air pressure is vital to ensure reproducible, quantitative data.

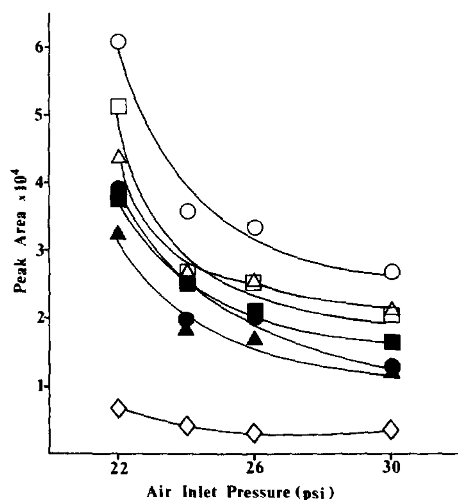

Fig. 8. Effect of detector air inlet pressure on the mass detector response to seven saturated triglycerides: 10.73 μg tripalmitin, △; 13.07 μg trimyristin, ○; 13.15 μg trilaurin, □; 12.33 μg tricaprin, ●; 11.74 μg tricaprylin, ▲; 13.08 μg tricaproin, ■; 12.61 μg tributyrin, ◇. Data Master integration.

Effect of solvent flow rate on detector response

As the detector response has been shown to vary with changes in evaporation conditions (*i.e.* changes in temperature or air pressure) it would be expected that changes in the solvent flow-rate would also affect the detector response. As modern microcomputer controlled chromatographs often permit not only the use of a solvent composition gradient, but also the use of a solvent flow-rate gradient it was important

to establish the effect of changes in solvent flow-rate on the response of the detector to triglycerides. The effect of solvent flow-rate on the response of the detector to tricaprin and trilaurin was determined by direct injection as is shown in Fig. 9. The effect of solvent flow-rate on detector response follows a sigmoidal curve, with increased flow-rates causing decreased responses for both tricaprin and trilaurin. It is important to note that the most marked decreases in response with increase in flow-rate (in the order of 30% for both triglycerides) are noted between 1.0 and 2.0 ml min^{-1}, the flow-rate range most commonly used for HPLC using standard width columns.

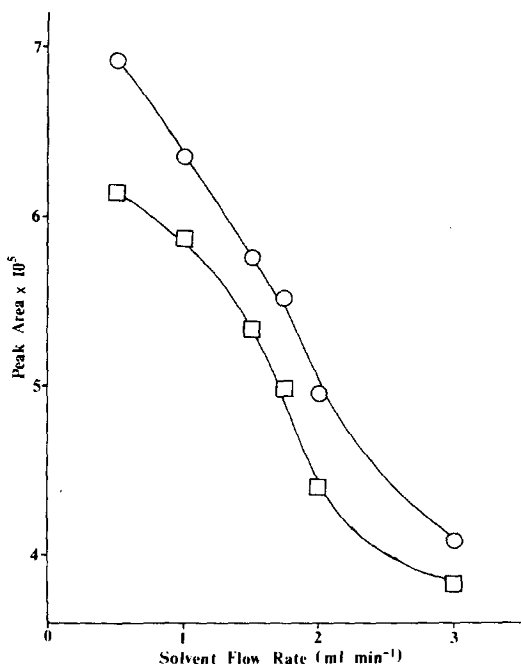

Fig. 9. Effect of solvent flow-rate (acetone) on mass detector response to two saturated triglycerides: 14.06 μg tricaprin, ○; 13.24 μg trilaurin, □. Hewlett-Packerd integration.

Solvent effects on detector response

The effect of solvent on the response of the detector to trilaurin and tricaprin was investigated, and the results are presented below in Table V. It is not easy to explain why these marked differenes in response should be observed. The trend is similar for both trilaurin (which is not readily soluble in all the solvents) and tricaprin which is freely soluble in all four solvents.

Charlesworth[12] has reported that the three main factors responsible for solvent evaporation (all other conditions being equal) are temperature difference, molar volatility (ΔH_v/MW, where ΔH_v is the enthalpy of vaporisation and MW is molecular weight) and initial droplet size. The temperature difference is constant, and it seems most probable that the molar volatility of the solvent and the solvent properties affecting the initial droplet size are the most significant factors in determining the detector response (*i.e.* the efficiency with which the solvent is evaporated).

TABLE V

DETECTOR RESPONSE TO TRILAURIN AND TRICAPRIN IN VARIOUS SOLVENTS

Solvent	*Molar volatility, $\Delta H_v/MW$*	*Response to tricaprin**	*Response to trilaurin**
Chloroform	1.1574	2613487	1804149
Acetone	1.1431	1060676	1113719
Acetonitrile	2.9935	755270	811747
2-Propanol	2.0184	704414	787010

* Values interpolated from a calibration curve, to correspond to 25 μg injected in 5 μl.

These marked differences in response depending upon the solvent composition have serious implications for the use of light-scattering detectors, based on these principles, for quantitative analyses under gradient elution conditions. An experimental detector working on similar principles has been reported to give no baseline drift with various non-aqueous solvent combinations[6], as is also the case with the mass detector. However, the data in Table V clearly indicate that a mere absence of base-line drift does not imply that gradient elution can necessarily be used where accurate quantitation is required.

The data in the section comparing reproducibility of determinations under both gradient and isocratic elution conditions demonstrate that reproducibility is poor under conditions of gradient elution, and particularly so if a steep solvent gradient is used. This is to be expected in view of the response data for the two triglycerides in acetone and acetonitrile. If any particular triglyceride was to elute in a slightly different solvent composition, as is frequently the case when ambient temperature fluctuations occur to a duplicate chromatographic run then the detector response could be expected to be slightly different, hence these errors would be added to any other errors inherent in the procedure.

Applications

An example of the use of the mass detector is presented in Fig. 10. It can be

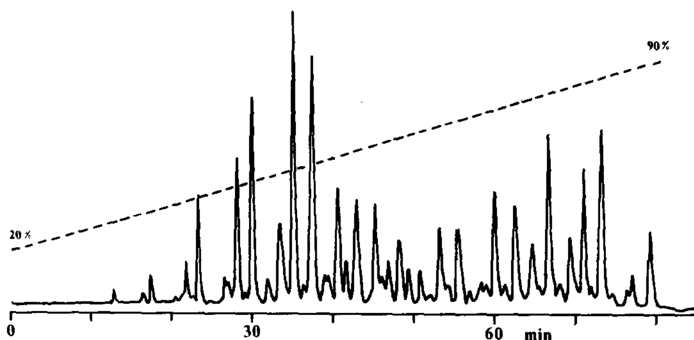

Fig. 10. Liquid chromatographic separation of butter triglycerides. Column: Spherisorb-5-ODS 2 (50 × 4.6 mm I.D. plus 250 × 4.6 mm I.D.). Mass detector settings: attenuation, × 1; photomultiplier, × 2; evaporator, 25; air, 22 p.s.i. Solvent gradient was linear from 20% to 90% acetone in acetonitrile at a rate of 1.5 ml min^{-1}.

seen from the chromatogram, that gradient elution with acetone and acetonitrile produces separation of many of the triglycerides which are present in the complex mixture of butter fat triglycerides. It has previously been shown, that these eluting solvents when used in conjunction with the mass detector, produce better chromatograms than with refractive index or UV detectors, as there are no problems with gradient or solvent incompatibility[11]. Hence the mass detector can be used to advantage in the analysis of complex triglyceride mixtures.

CONCLUSION

Detection limits for non-volatile triglycerides are acceptable for the mass detector and compare well with detection limits of refractive index detectors but are too high for volatile materials such as tributyrin and FAMEs. The mass detector is not mass responsive and the response is not linear for triglycerides, although logarithmic plots of response against amount injected approximate to straight lines for triglycerides. The detection limit of the mass detector for triglycerides is of the same order as its detection limit for sugars.

The response of the detector to triglycerides and FAMEs was reduced by increased atomiser inlet pressures and varied with change in evaporation temperature. Increases in solvent flow-rate caused marked decreases in detector response, particularly in the range 1.0–2.0 ml min^{-1}. In addition the response of the detector varied for triglycerides depending upon the composition of the eluting solvent.

Reproducibility is dependent on chromatographic conditions. For isocratic elution it is acceptable with coefficients of variation of less than 6% for the saturated and unsaturated triglycerides studied, except for tributyrin which is very volatile. For gradient elution reproducibility was poor, probably due to the effects which changes in solvent composition would have on the response.

In view of these influences on the response of the detector to triglycerides it is concluded that, the mass detector is only suitable for quantitative analysis of lipids, under conditions of isocratic elution, provided that a calibration is carried out for the materials to be analysed, under exactly the same conditions of temperature, pressure solvent flow-rate and composition as when the analyses are carried out. Furthermore, it is essential that a clean air supply is provided and that adequate provision is made for the toxic exhaust fumes.

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