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Quantitative estimation of garlic oil content in garlic oil based health products

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The effective carbon number (ECN) concept can estimate FID response factors of garlic sulphides with good accuracy and the incorporation of such factors is necessary for the precise quantitative analysis of garlic sulphides. A GLC procedure using C_{18} cartridge liquid solid phase extraction was developed to analyse sulphide content in garlic oil based health products. Diallyl disulphide, allyl methyl trisulphide and diallyl trisulphide were found to be the three major sulphides, contributing about 600 mg g^{-1} in the oil. The garlic oil based health products examined in this investigation had a very similar sulphide profile to that of pure garlic oil. The total garlic oil content in these products may thus be estimated by relating individual sulphide weight per cent in the products to their composition in garlic oil. Such an estimation was found to be useful and reliable in order to check the manufacturer's label claims of garlic oil contents.

INTRODUCTION

Garlic has been recognised since ancient times as a flavouring agent for food but also for its medicinal properties. Over recent years, a variety of garlic based health products has become available on the market. The claimed health advantages of these garlic products has generated a great interest in and a need for analytical methods to evaluate their quality.

The prime active component in garlic and its health products is suggested to be allicin but also involved are its degradation products, a range of sulphides. The intact garlic bulb is odourless, but when it is cut and/or homogenized, the active enzyme system alliinase converts alliin (*S*-allyl-L-cysteine sulphoxide) into allicin (diallyl thiosulphinate) (Stoll & Seebeck, 1948). Allicin is not a stable compound and readily degrades via several pathways to form the secondary products of various sulphides (Brodinitz *et al.*, 1971; Block, 1985), contributing the characteristic flavour and odour of garlic.

HPLC methods have been reported for the analysis of garlic products (Jansen *et al.*, 1987; Mochizuki *et al.*, 1988; Iberl *et al.*, 1990). The advantage of HPLC analysis is that the method utilises no heat and is unlikely to cause thermal damage to any compound in garlic products during analysis. However, the relatively low resolution and lack of universal detection system for individual sulphides limit the use of the method, and therefore it is mainly used for the analysis of alliin and allicin.

GC methods have also been proposed for the analysis of secondary sulphides in garlic products. Several studies have reported the use of packed column GC (Saito *et al.*, 1989) and, more recently, capillary column GC (Yu & Wu, 1989) and various sulphides have been identified as being present in garlic oil (Laakso *et al.*, 1989; Yu & Wu, 1989). However, these studies mainly investigated the profiling of garlic sulphides and their identification using GC/MS. The quantitative analysis has not been thoroughly studied. For example, garlic oil is normally formulated with vegetable oil in health products and research is needed to investigate the recovery of garlic sulphides before GC analysis.

The profiling of garlic normally uses a flame ionisation detector to determine their contents. The FID response of hydrocarbons is generally proportional to the mass of carbon present in the compounds. Compounds that contain, for example, oxygen, nitrogen, halogens or sulphur give varied responses. Further research is needed to determine/predict FID response factors for garlic sulphides and to develop a quantitative method to incorporate these factors in order to assess the quality of garlic products more accurately.

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This investigation reports the development of a commercially viable GLC method for the analysis of garlic oil based health products.

MATERIALS AND METHODS

Material

Sulphide standards, diallyl sulphide (purity 97%), diallyl disulphide (purity 80%), dimethyl disulphide (purity 98%), and dipropyl disulphide (purity 99%) were purchased from Fluka Chemicals Ltd; and allyl methyl sulphide (purity 98%), diethyl disulphide (purity 99%) and dibutyl disulphide (purity 98%) were obtained from Aldrich Chemical Company Ltd. Garlic oil was supplied by Seven Seas Health Care Ltd, Hull, UK.

GLC analysis

A Perkin-Elmer 8420 GC with DBwax column (30 m × 0·32 mm, film thickness 5 μm) was used for the analysis. The carrier gas was helium at 22·5 psig. An on-column injection mode was used with an injection volume of 0·2 μl. The following temperature programme was used:

$$55°C\ (10\ min) \xrightarrow{2°C\ min^{-1}} 150°C \xrightarrow{15°C\ min^{-1}} 220°C\ (10\ min)$$

Sample preparation for garlic oil

About 20 mg garlic oil was accurately weighed into a sample bottle and 2 ml iso-octane (containing 2 mg dipropyl disulphide as internal standard for quantitative estimation) was then added.

Sample preparation for garlic oil based health products

Direct acetonitrile extraction

Three grams of garlic oil based health products (or sunflower oil and garlic oil mixture in the study of method development) were extracted with 3 ml portions of acetonitrile several times. 0·1 ml (10 mg ml^{-1}) internal standard (dipropyl disulphide) was then added to each acetonitrile extract and they were centrifuged at 5 000 *g* for 10 min to separate residual (sunflower) oil in the extracts before GC analysis.

Solid phase extraction

Garlic oil based health products (or sunflower oil and garlic oil mixture in the recovery study) (0·8 ml) were directly applied to a C_{18} solid phase cartridge (Waters). Sulphides were eluted with 3 ml acetonitrile and 0·1 ml (10 mg ml^{-1}) internal standard (dipropyl disulphide) was then added to the eluate. The eluate was centrifuged for 10 min at 5 000 *g* (to remove any sunflower oil residue in the eluate) and 0·2 μl supernatant was directly injected into the GC for analysis.

RESULTS AND DISCUSSION

Prediction of FID response factor for garlic sulphides

The concept of effective carbon number (ECN) is frequently used in the prediction of FID response factors in GC analysis. The systematic prediction of FID response factors for sulphides has not been reported although FID is commonly used to determine the profile of sulphides in garlic oil quantitatively. In this investigation, it was found that the number of sulphur atoms present does not change the ECN of garlic sulphides (Table 1). Since dipropyl disulphide is most commonly used as an internal standard to determine garlic sulphide content, the relative FID response factors of garlic sulphides to this internal standard are calculated and listed in Table 1. This prediction was verified using several sulphides (Table 1).

The values determined showed good agreement with those predicted. If RRF is not incorporated into the analysis of garlic sulphides, the sulphide profiling can-

Table 1. Relative FID response factor of garlic sulphides to dipropyl disulphide

Sulphide	Molecular weight	Carbon number	RF	RF determined	Error in prediction (%)
Dipropyl disulphide	150	6	1·000	1·000	0·00
Dimethyl disulphide	92	2	1·840	1·772	−3·70
Diethyl disulphide	122	4	1·220	1·187	−2·70
Dibutyl disulphide	178	8	0·890	0·899	1·01
Diallyl sulphide	114	6	0·760	0·783	3·03
Allyl methyl sulphide	88	4	0·880	0·899	2·16
Diallyl disulphide	146	6	0·973		
Allyl methyl disulphide	120	4	1·200		
Dimethyl trisulphide	126	2	2·520		
Diallyl trisulphide	178	6	1·187		
Allyl methyl trisulphide	152	4	1·520		
Allyl propyl disulphide	148	6	0·987		

NB: results were means of three determinations. All the results had a standard deviation of less than 1%.

not be accurately determined and the sulphide content may be underestimated, since most garlic sulphides have a RRF greater than 1.

Various sulphur containing compounds have been reported to be present in garlic oil (Yu & Wu, 1989; Iberl *et al.*, 1990). In this study, eight sulphides have been positively identified in samples of garlic oil. Table 2 illustrates their weight contents and RRF. The total sulphide content increased from 650 to 797 mg g^{-1} when RRF was incorporated into the calculation. The three major sulphides (diallyl disulphide, allyl methyl trisulphide and diallyl trisulphide) contribute nearly 600 mg g^{-1} increase compared with the uncorrected content. This clearly indicates the importance of incorporating the RRF into the calculation of garlic sulphides.

Development of a method for garlic oil products

In the analysis of garlic oil based health products, one major difficulty is the sample preparation. There are several reports investigating the profiling of garlic oil using either GC or HPLC. However, these studies mainly concentrate on the qualitative identification of garlic sulphur containing compounds or some quantitative investigation by exploring the relative percentage of these sulphides in garlic oil. Transfer of the analytical method for pure garlic oil to health products (which are complex mixtures) needs considerable modification. A sample preparation method to extract the active sulphur containing compounds from the product mixture is required.

In the garlic based health product market, one of the major formulations is oil based capsules. To extract garlic sulphides from the product oil mixture (normally garlic oil/vegetable oil), one commonly used procedure is direct polar solvent extraction using acetonitrile. When garlic oil and vegetable oil were mixed in a ratio of 15 mg (garlic oil) to 3 g (vegetable oil) and extracted with a polar solvent (acetonitrile), it was found that the first extraction only recovered about 30–40% of the sulphides with the rest still remaining in the vegetable oil. Moreover, recoveries varied with different sulphides. For example, dimethyl trisulphide and diallyl sulphide had the highest recovery (about 40%), while the two main sulphides, diallyl trisulphide and allyl methyl trisulphide, had a recovery of only about 30%. Up to five extractions were necessary to achieve a near complete recovery. Consequently, in the sample preparation for this analysis of oil based products, the sample was extracted five times with acetonitrile and all the extracts were combined and any internal standard added following this but before GC analysis. This sample preparation method provides a reasonable recovery of sulphides from oil mixtures and can therefore be used for the analysis of oil based products. However, the method was very time consuming and in view of this, an alternative preparation method was developed.

Solid phase extraction is quite commonly used in sample preparation for GC analysis to remove contaminants before injection on to a GC column. In this present study, it was found that solid phase extraction could be very useful in the sample preparation of the oil based products. Several types of solid phase extraction cartridges (silica and C_{18}) were tried. It was found that the direct application of an oil mixture (0·8 ml) to C_{18} cartridge followed by eluting with (3 ml) acetonitrile was very efficient in recovering sulphides from garlic oil and vegetable oil mixtures. A recovery of more than 90% can be achieved with this easy technique (Table 3). The method can be used for the analysis of oil based garlic products and the reported results of analysis in this study were obtained using this method.

Table 2. Sulphide contents of garlic oil

	RRF	Corrected weight content (mg g^{-1})	% Change to uncorrected weight content
1. Dimethyl disulphide	1·840	33·9	+84·3
2. Diallyl sulphide	0·760	23·0	−23·8
3. Allyl methyl disulphide	1·200	82·9	+20·0
4. Dimethyl trisulphide	2·520	60·0	+152·0
5. Diallyl disulphide	0·973	186·7	+2·7
6. Allyl methyl trisulphide	1·520	190·9	+52·0
7. Allyl propyl trisulphide	1·205	24·5	+2·1
8. Diallyl trisulphide	1·187	195·2	+18·7
Total sulphides		797·1	

The weight content was calculated as follows:

$$\text{corrected weight content (mg g}^{-1}) = \frac{A_{\text{sample sulphide}} \times W_{is}}{A_{is} \times W_{\text{sample}}} \times 1000 \times \text{RRF}$$

where $A_{\text{sample sulphide}}$ = peak area of sample sulphide; A_{is} = peak area of internal standard; W_{sample} = sample weight (mg); and W_{is} = weight of internal standard (mg).

Table 3. Percentage recovery of sulphides from garlic oil and vegetable oil mixture using C_{18} cartridge

	Recovery (%)
Dimethyl disulphide	114 (9.0)
Diallyl sulphide	88 (4.9)
Allyl methyl disulphide	94 (3.6)
Dimethyl trisulphide	90 (4.5)
Diallyl disulphide	94 (5.3)
Allyl methyl trisulphide	88 (4.0)
Allyl propyl trisulphide	92 (8.7)
Diallyl trisulphide	91 (6.2)

NB: results are means of three experiments and figures in parathensis are standard deviations.

Garlic oil based health products

Several garlic oil based products have been analysed for their sulphide profile and content and the results are listed in Table 4. In general, the products have a similar profile of sulphides to that of pure garlic oil and the three major sulphides were again found to be diallyl disulphide, allyl methyl trisulphide and diallyl trisulphide. Similar results have also been reported in other studies (Lawson *et al.*, 1991). The garlic oil content in the products may thus be estimated by assuming that the composition of the oil is the same as in the pure garlic oil (very likely to be true) and the garlic oil content can thus be calculated by the content of a particular sulphide and its weight percentage related to that in the pure garlic oil (Table 4). All the results were corrected with RRF. The value for any single sulphide can be used to calculate the total garlic oil content but it is recommended that values for the three major sulphides, diallyl di- and trisulphides and allyl methyl trisulphide are used to give a more accurate average result. When the three major sulphides were chosen to calculate the garlic oil content of the products, it was found that the calculated garlic oil content of the examined garlic products was very similar to the manufacturer's claimed values of garlic oil content in the products.

CONCLUSION

The FID response factors of garlic sulphides can be predicted using the effective carbon number concept and the incorporation of a relative response factor to an internal standard (dipropyl disulphide) was necessary in the quantitative analysis of garlic sulphides. The developed GC method using C_{18} cartridge solid extraction as the sample preparation procedure can provide a rapid and reliable method applicable in most laboratories for the confirmation of claims of garlic oil content of products.

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Table 4. Garlic oil content and sulphide composition of garlic oil based health products

	Product A	Product B	Product C
Sulphide profile (mg g^{-1} of sample (filling))			
Dimethyl disulphide	0·046 (0·007)	0·128 (0·007)	
Diallyl sulphide	0·028 (0·010)	0·077 (0·005)	
Allyl methyl disulphide	0·138 (0·005)	0·412 (0·029)	0·036 (0·008)
Dimethyl trisulphide	0·111 (0·020)	0·254 (0·010)	
Diallyl disulphide	0·340 (0·025)	0·924 (0·019)	0·087 (0·021)
Allyl methyl trisulphide	0·324 (0·024)	0·844 (0·071)	0·068 (0·004)
Allyl propyl trisulphide	0·045 (0·016)	0·066 (0·002)	
Diallyl trisulphide	0·282 (0·004)	0·814 (0·013)	0·119 (0·011)
Estimated garlic oil content (%) (based on the following sulphides)			
Diallyl disulphide	0·18	0·55	0·046
Allyl methyl trisulphide	0·17	0·44	0·036
Diallyl trisulphide	0·19	0·53	0·049
mean	0·18	0·51	0·045
Label claim garlic oil content (%)	0·2	0·53	0·06

NB: results are means of three determinations and figures in parathensis are standard deviations.

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