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Saponins in asparagus — potential for waste stream utilisation

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A report prepared for
Asparagus Research Council

Copy 6 of 6

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Contents

1 Executive summary

This report describes the results of an initial project undertaken for the New Zealand Asparagus Research Council to investigate saponins in asparagus (*Asparagus officinalis*). The first part of the report is a literature review of saponins, their potential health benefits and distribution in asparagus. The second part describes the results of some preliminary analyses to determine if we can detect saponins in New Zealand-grown asparagus and the potential for waste stream utilisation.

Although saponins were originally used as natural detergents and fish poisons they are now regarded as a medically important group of natural products. They have a wide range of biological activities (e.g. haemolytic, anti-inflammatory, anti-microbial, anti-mitotic, molluscicidal, cytotoxic, cardioprotective, cholesterol lowering and anti-cancer). Although many of these activities are beneficial, saponins are also classed as antinutrients. Based on the scientific literature it may be possible to extract saponins from plant material for use directly as health beneficial compounds or for synthesis into other drugs. Saponins have been reported in various members of the *Asparagus* genus, including the common vegetable asparagus, *A. officinalis*.

Our preliminary investigation did not show significant quantities of saponin in the lower, white section of the spears of one asparagus cultivar (UC157), although they have been reported in this cultivar in the literature. However, saponins were detected in whole spears of three cultivars (Jersey Giant, Pacific Purple and UC157). Further investigation is required to confirm if there is any potential to extract saponins from asparagus waste streams.

2 Background

A preliminary review of the potential health benefits of asparagus has been conducted by Lister (2001). Asparagus extracts have been shown to possess a range of biological activities including antifungal, antimutagenic, diuretic, cytotoxic, antiviral, molluscicide and antioxidant. Our previous studies have focused on the antioxidant activity and phenolic (flavonoid) components of asparagus (Lister 2001, 2002). However, some reports indicate that saponins are major components of asparagus and suggest they are the active principle responsible for many of the biological activities attributed to asparagus (Goryanu et al. 1976a; Sharma et al. 1983; Shao et al. 1996).

This report covers, firstly, a review of the scientific literature on saponins, their health benefits and distribution and, secondly, provides a preliminary analysis of the saponins in asparagus.

3 Literature review

3.1 Saponins

Saponins are a large, widely distributed group of plant substances named from their ability to form strongly foaming, soap-like solutions with water. They are divided into three major groups: triterpenoid, basic steroid and steroid saponins. They have been detected in over 70 plant families (Tschesche & Wulff 1973). Sapogenins are the aglycones of saponins and lack the sugar group attached to them. This may be lost due to enzymatic or acid hydrolysis or sapogenins may occur in nature (Agrawal et al. 1985).

Although saponins were originally used as natural detergents and fish poisons they are now regarded as a medically important group of natural products and they have a wide range of biological activities (Price et al. 1987). Although many of these activities are beneficial, saponins are also classed as antinutrients. They may reduce the availability of some nutrients and inhibit growth (Thompson 1993). Although not particularly toxic to humans if taken orally, they are toxic to cold blooded animals (Oketch-Rabah 1998).

Some biological activities of saponins include:

1. **Haemolytic activity**: Only some saponins (a few in the class of oleanic saponins) cause haemolysis of red blood cells and this effect is restricted to particular types; monodesmosidic saponins are more haemolytic than bidesmosidic saponins (Balansard & Bornard 1966, cited in Ahmad et al. 1996; Romussi et al. 1979, cited in Ahmad et al. 1996).
2. **Anti-inflammatory activity**: Some triterpenoid saponins from *Tetrapanax papyriferum* have been reported to have anti-inflammatory activity, and saponins from *Panax notoginseng* have been shown to be effective against several experimental models of inflammation in mice and rats (Sugishita et al. 1982; Hao & Yang 1986).
3. **Anti-microbial activity**: Members of the saponin family are generally good anti-fungal and anti-bacterial compounds (Grover & Rao 1988; Shimoyamada et al. 1990, 1996). The saponins are more effective than the sapogenins and acylated saponins (Tachescho et al. 1966, cited in Ahmad et al. 1996; Schlosser 1971, cited in Ahmad et al. 1996). Some saponins have also been shown to be effective against HIV *in vitro* (Lasztity et al. 1998).
4. **Anti-mitotic activity**: Certain saponin glycosides and their magnesium salts from *Hedra helix* have been shown to inhibit the growth of certain

cells without destroying them (Kupchan et al. 1967, cited in Ahmad et al. 1996).

5. **Molluscicidal activity**: One particular class of saponins (oleananes) has been shown to have molluscicidal activity (Parkhurst et al. 1973), and may control certain parasitic diseases by killing the host (Riccio et al. 1987, cited in Ahmad et al. 1996).

6. **Cytotoxic activity**: Some saponins have been shown to kill certain cell types (Anisimov et al. 1980, cited in Ahmad et al. 1996; Shao et al. 1997).

7. **Action on the cardiovascular system**: Cardiotonic activity has been observed and beneficial effects of certain saponins have been noted in humans and rabbits suffering from arteriosclerosis (Guseinov & Iskenderov 1972, cited in Oketch-Rabah 1998).

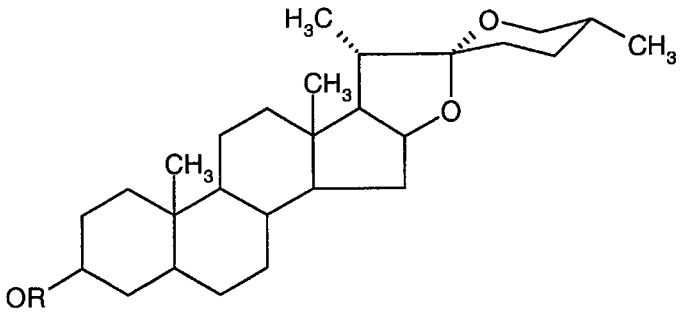
8. **Effects on serum cholesterol levels**: Various researchers have noted that saponins, especially those of oleanic acid, may reduce serum cholesterol (Lutomski 1983; Price et al. 1987; Lasztity et al. 1998). Thus, consumption of saponins may provide a useful means of controlling plasma cholesterol in man (Ahmad et al. 1996).

9. **Anti-cancer**: Certain saponins may have a role in cancer protection (Steinmetz & Potter 1996).

Some plant saponins are used as raw materials in the biosynthesis of biologically active steroid compounds of medicinal importance. Examples include Diosgenin from *Dioscorea* species, which is also found in *Asparagus adscendens* (Sharma et al. 1980) and is used as a starting material in the partial synthesis of the contraceptive oestrogen and corticosteroids (Ahmad et al. 1996). Another example is hecogenin from *Agave* species, which is used as a starting material in the partial synthesis of corticosteroids (Evans 1989). Both compounds are sapogenins. Some triterpenoid saponins are also of economic interest because of their sweet taste, e.g. glycyrrhizin from liquorice roots (Evans 1989).

3.2 *Saponins in the genus* Asparagus

The genus Asparagus is cited as medicinally important, largely because both the aerial and underground parts are rich in saponins and sapogenins. The only class of saponin reported in the genus *Asparagus* is the steroidal saponins. The saponins that are of interest in this genus may be classified as spirostanes, furostanes and furospirostanes (Figure 1).



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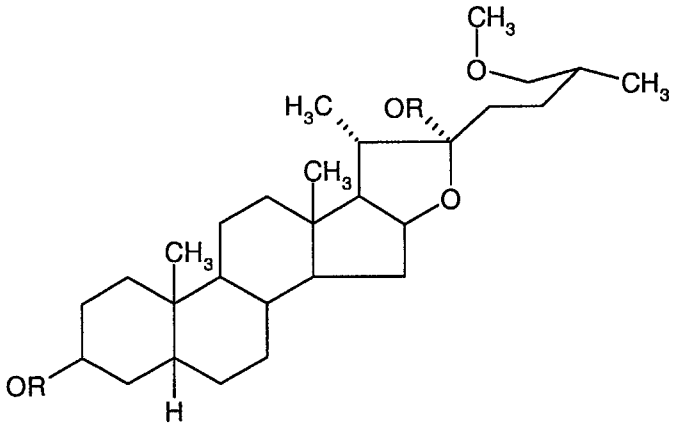
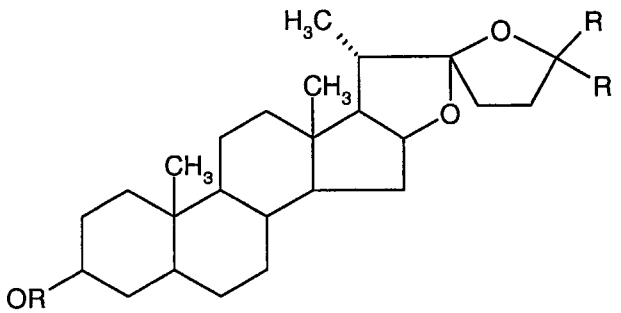


furostane skeleton



furospirostane skeleton

Figure 1: Structure of the main groups of steroidal saponins in the genus Asparagus *(adapted from Oketch-Rabah 1998).*

A review of phytochemical studies on *Asparagus* species (published up to 1994) has been conducted by Ahmad et al. (1996) and a slightly more recent review by Oketch-Rabah (1998). The composition of saponins in some species of asparagus has been investigated, including *Asparagus adscendens* (Sharma & Sharma 1984; Tandon et al. 1990), *A. africanus* (Debella et al. 1999), *A. curillus* (Sharma et al. 1983; Sharma & Sharma 1993), *A. dumosus* (Ahmad et al. 1998; Khaliq-uz-Zaman et al. 2000), *A.*

filicinus (Sharma & Thakur 1994, 1996), *A. meicoclados* (Feng et al. 2002), *A. officinalis* (the common cultivated asparagus, see below), *A. plumosus* (Sati & Pant 1985; Paczkowski et al. 1990), *A. racemocus* (Chifundera et al. 1993) and *A. sprengeri* (Sharma et al. 1983). Details of *A. officinalis* are given in the following section and a summary of the names of specific compounds reported in different species can be found in Ahmad et al. (1986) and Oketch-Rabah (1998).

There are also a few reports on the biological activities of these saponins in the asparagus family. Reports include anti-nociceptive and anti-inflammatory activity (Nwafor & Okwusaba 2003), spermicidal activity (Pant et al. 1988) and the ability to inhibit the growth of some fungi (Shimoyamada et al. 1990). Further activities are discussed in Section 3.2.1.

3.2.1 *Saponins from* Asparagus officinalis

Saponins are reported to be responsible for the bitter taste of asparagus, especially in the butt of the spear (Shimoyamada et al. 1996). A number of different saponins have been reported in different parts of *A. officinalis*, although in many cases they have not been fully identified. Goryanu et al. (1976 a, b, 1977) have identified asparagosides A, B, C, E, F and H. Two oligofurostanosides in asparagus seeds were identified as methyl protodioscin and protodioscin (Shao et al. 1997). A series of spirostane glycosides in which the aglycone is sarsapogenin and the simplest glycoside is a monoglucoside has been reported by Paczkowski & Wojciechowski (1988) in asparagus leaves. Other compounds noted have been other asparagosides, asparasaponin I and II, and furostanol asparagosides B, E, G, and H (Ahmad et al. 1996).

The crude saponins from the shoots and seeds of *A. officinalis* have been shown to have antitumor activity; they inhibited the growth of human leukaemia cells in culture in a dose and time-dependent manner (Shao et al. 1996, 1997). The 'bottom cut' of white asparagus has been investigated as a potential source of antifungal saponins (Shimoyamada et al. 1990).

4 Analysis of saponins in New Zealand asparagus samples

4.1 Aim

The aim of this part of the project was to test a Crop & Food Research methodology, developed for peas, to determine whether it could be used to detect saponins in asparagus. In particular, we were interested in exploring the potential to extract saponins from the lower section of spears, creating value from asparagus waste streams.

4.2 Methods

Methods used were based on those developed for analysing pea saponins. Freeze-dried asparagus powder was extracted with ethanol:water. This

asparagus extract was dried, resuspended in water and cleaned up using a C18 column (water to wash and methanol to elute). The samples were then resuspended in 5 ml of methanol, sonicated and analysed by HPLC (Waters 600E system controller, Waters 717 Plus auto sampler @ 15°C and a reversed phase C18 column @ 25°C) and an ELSD detector (PL-ELS1000). The solvent system used is given in Table 1. Soyasaponin I (isolated from peas by Crop & Food Research staff) was used as a standard.

Four samples were analysed:

- Jersey Giant, whole spears
- Pacific Purple, whole spears
- UC157, whole spears
- UC157, white bottom (largely below ground section).

Table 1: Solvent gradient used for HPLC analysis of asparagus saponins.

Time (min)	Water (%)	Methanol (%)
0	70	30
2	30	70
4	30	70
6	0	100
22	0	100
23	70	30

4.3 Results and discussion

The HPLC traces of the four asparagus samples analysed are given in the Appendix. In three of the samples tested we detected at least one saponin, which appeared to match the standard (soyasaponin I). Other peaks were also detected, at least some of which may be saponins. The level of soyasaponin I in the three whole spear samples was very similar (Table 2). However, the white bottoms of cultivar UC157 contained no detectable saponins. Chin et al. (2002) have reported significant variations in saponin levels between genotypes. Shimoyamada et al. (1990) have reported saponins in the 'bottom cut' of white asparagus. However, they did not report the levels of saponins present or make any comparison with whole spears. We may need to modify our extraction, i.e. use different solvents and/or larger amounts of the bottom fraction, in order to detect the saponins.

Although soyasponin I has not been reported in asparagus, it is possible that this has a similar retention time to other saponins. Unfortunately we did not have enough standard to spike peaks and make more detailed investigation. One of the difficulties in this research area is obtaining standards. None are currently available for asparagus as far as we are aware. Further

investigation and trialling of different methods is required. Full identification of compounds would also require collection of NMR or MS data.

Table 2: Relative amounts of soyasaponin in asparagus samples.

Sample	Peak area (10^3) per gram
Jersey Giant, whole spear	22
Pacific Purple, whole spear	21
UC157, whole spear	19
UC157, white bottom	Not detected

5 Conclusions

Although saponins were originally used as natural detergents and fish poisons, they are now regarded as a medically important group of natural products and have a wide range of biological activities. Although many of these activities are beneficial, saponins are also classed as antinutrients. Based on literature reports, potential exists to extract saponins from plant material for their use directly as beneficial health compounds or for use in the synthesis of other drugs. Saponins have been reported in various members of the *Asparagus* genus, including the common vegetable asparagus, *A. officinalis*.

Our preliminary investigation did not show significant quantities of saponin in the lower white section of the spears of one asparagus cultivar (UC157), although they have been reported in the literature. However, saponins were detected in whole spears of three cultivars (Jersey Giant, Pacific Purple and UC157). Further investigation is required of the potential to extract saponins from asparagus waste streams.

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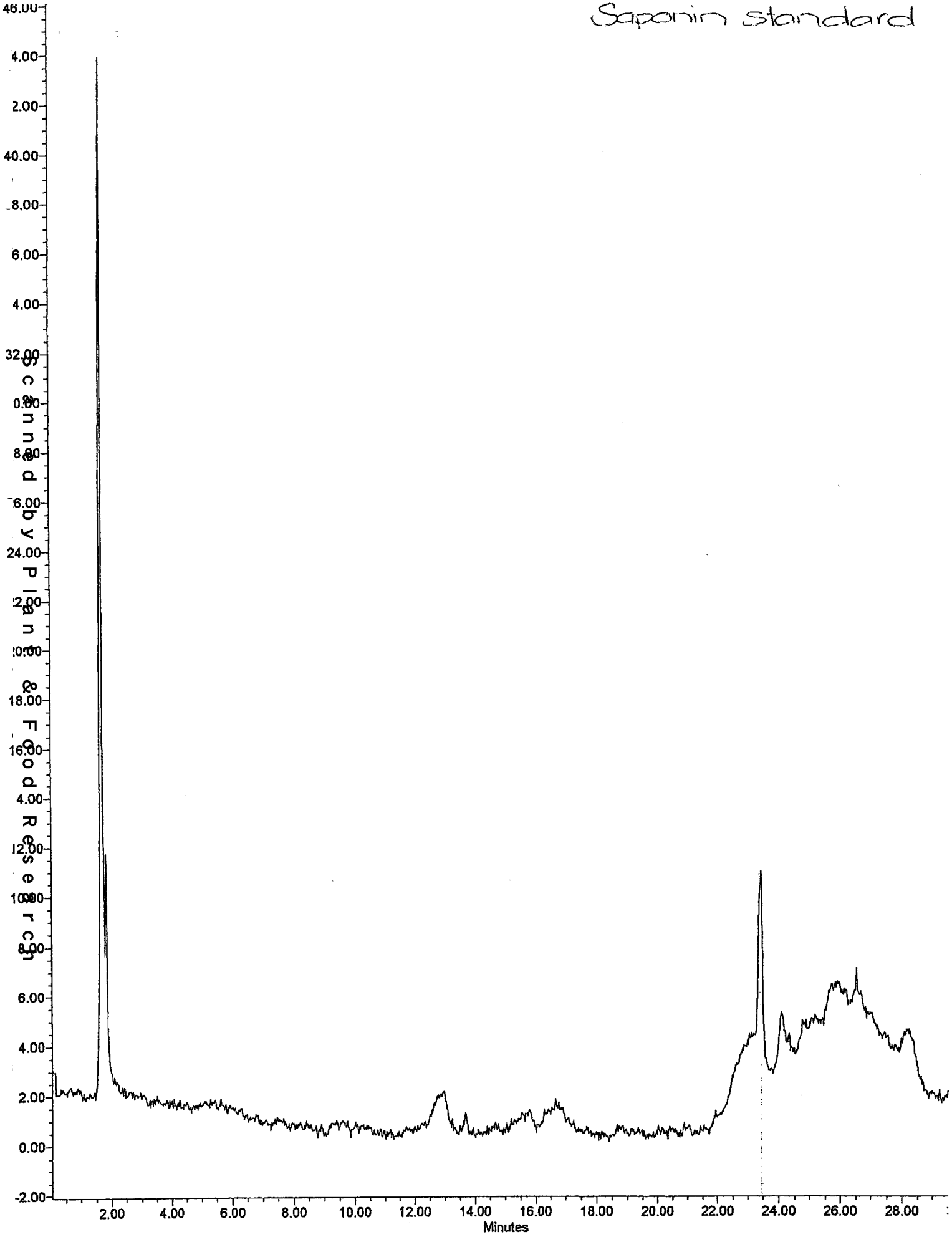
Appendix I HPLC traces of asparagus saponins

Saponin standard



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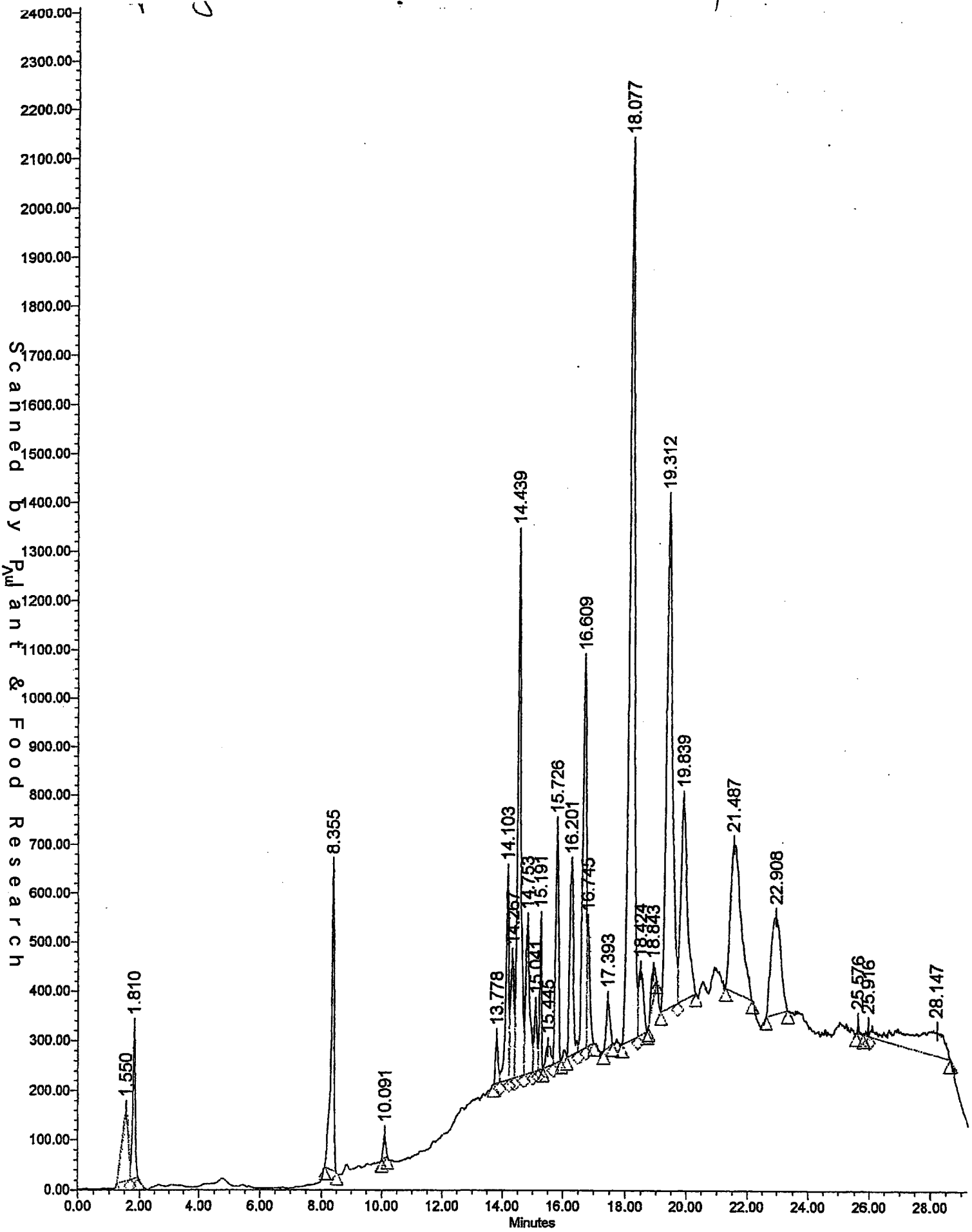
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18.077
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Minutes
Scanned by Plant & Food Research

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User Name System
Software Version 3.20

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Current Date 7/3/03

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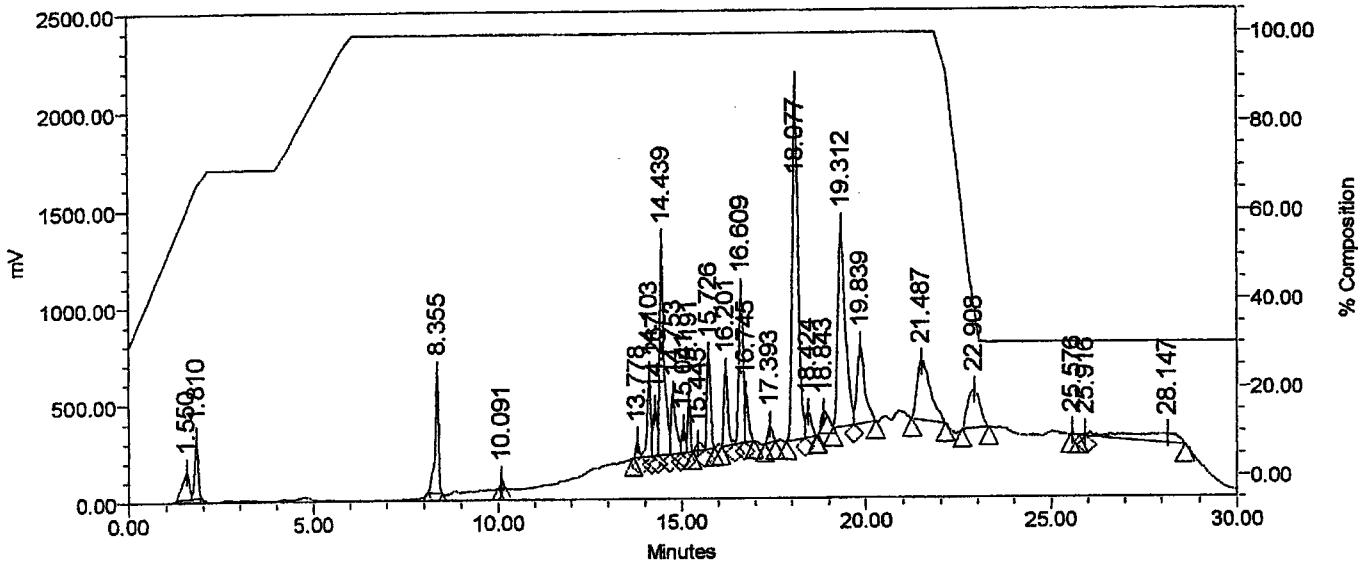

Peak Results

	Name	RT	Area	Height	Amount	Units
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2		1.810	1850468	305914		
3		8.355	4074069	616725		
4		10.091	249438	45804		
5		13.778	542585	92090		
6		14.103	2789654	421736		
7		14.267	1851279	243149		
8		14.439	8872760	1112319		
9		14.753	2904134	307946		
10		15.041	858072	129752		

	Name	RT	Area	Height	Amount	Units
11		15.191	1343110	303863		
12		15.445	489423	41171		
13		15.726	3554731	483210		
14		16.201	3143765	382338		
15		16.609	6230066	795533		
16		16.745	1612437	249161		
17		17.393	748707	96303		
18		18.077	19077899	1830439		
19		18.424	1416757	132574		
20		18.843	515995	61519		
21		19.312	14484447	1035156		
22		19.839	6052429	407926		
23		21.487	7348914	298942		
24		22.908	4444331	197340		
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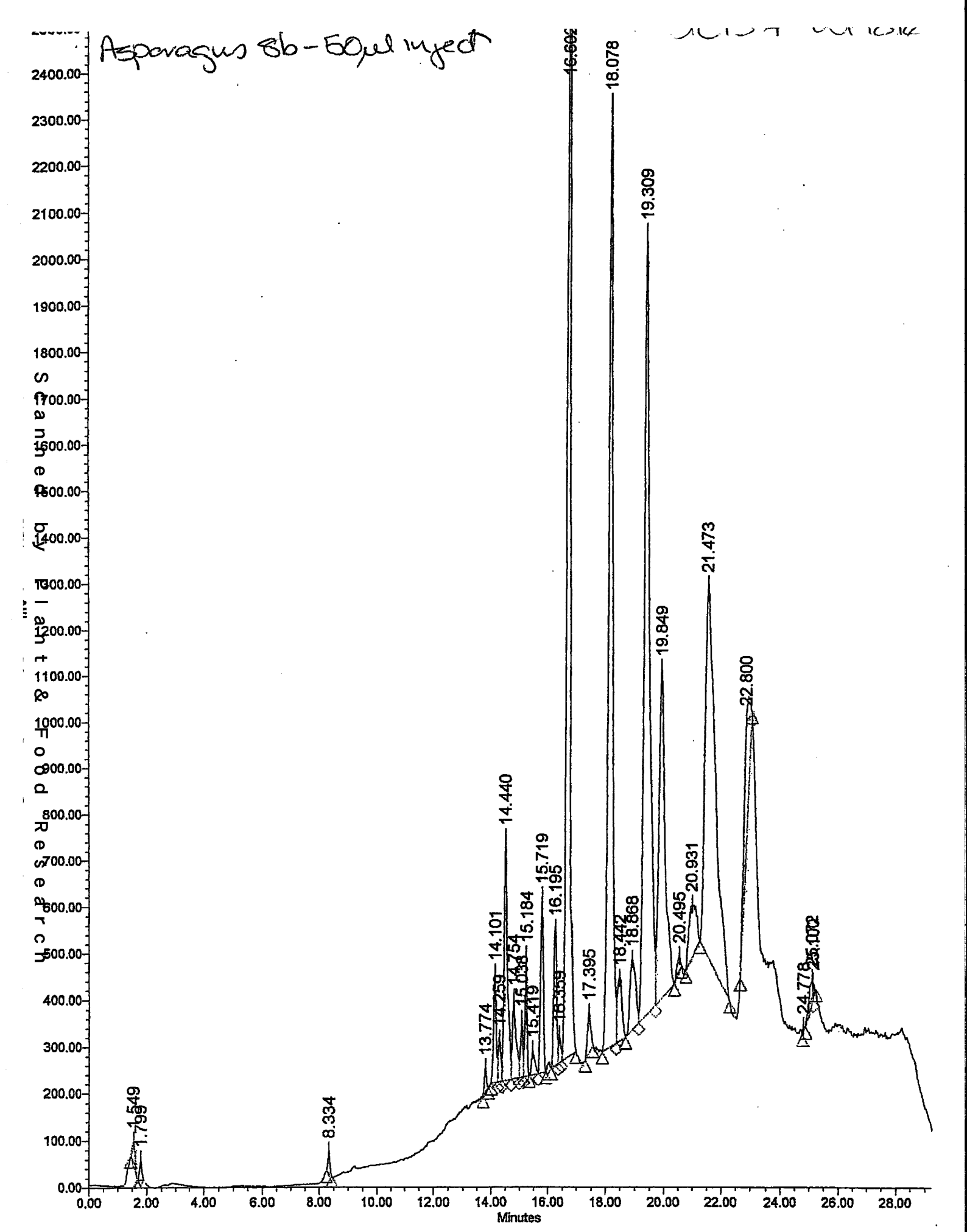
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Scanned by Plant & Food Research
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24.778

Project Name JOCELYN
User Name System
Software Version 3.20

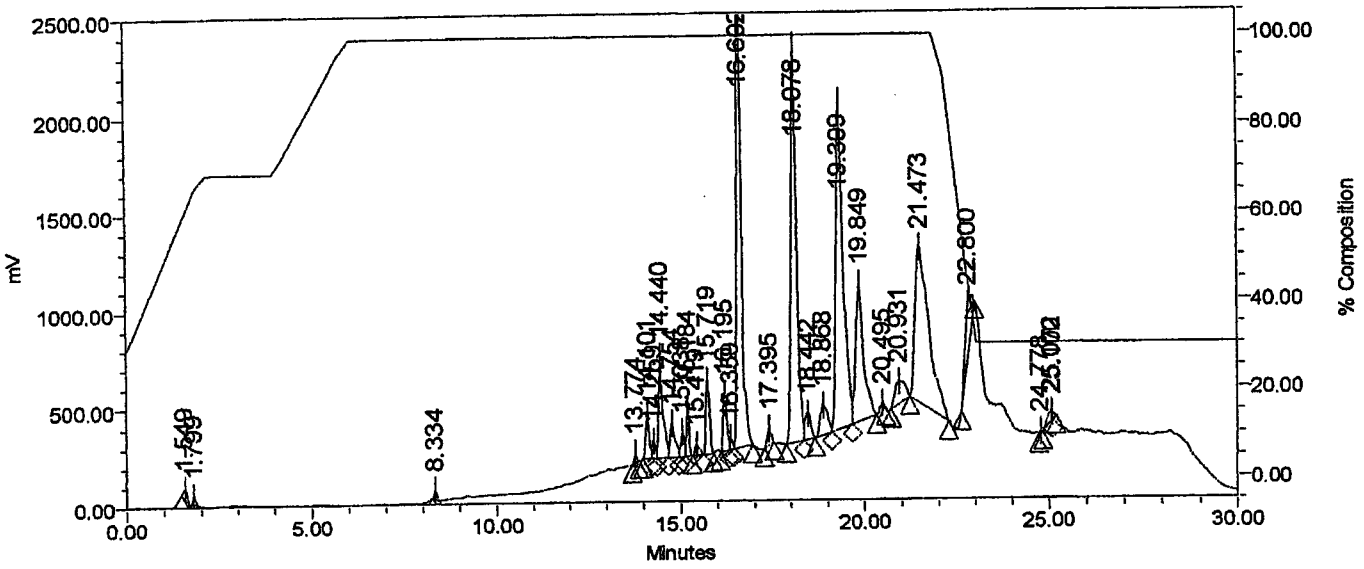
Report Method Name saponin
Current Date 7/3/03

Sample Information

SampleName asparagus 8b
Vial 5
Injection 1
Injection Volume 50.00 ul
Channel SATIN
Run Time 30.0 Minutes

Sample Type Unknown
Date Acquired 5/8/03 2:22:48 PM
Acq Method Set Saponin elsd
Processing Method saponin
Date Processed 7/3/03 4:29:17 PM

Auto-Scaled Chromatogram

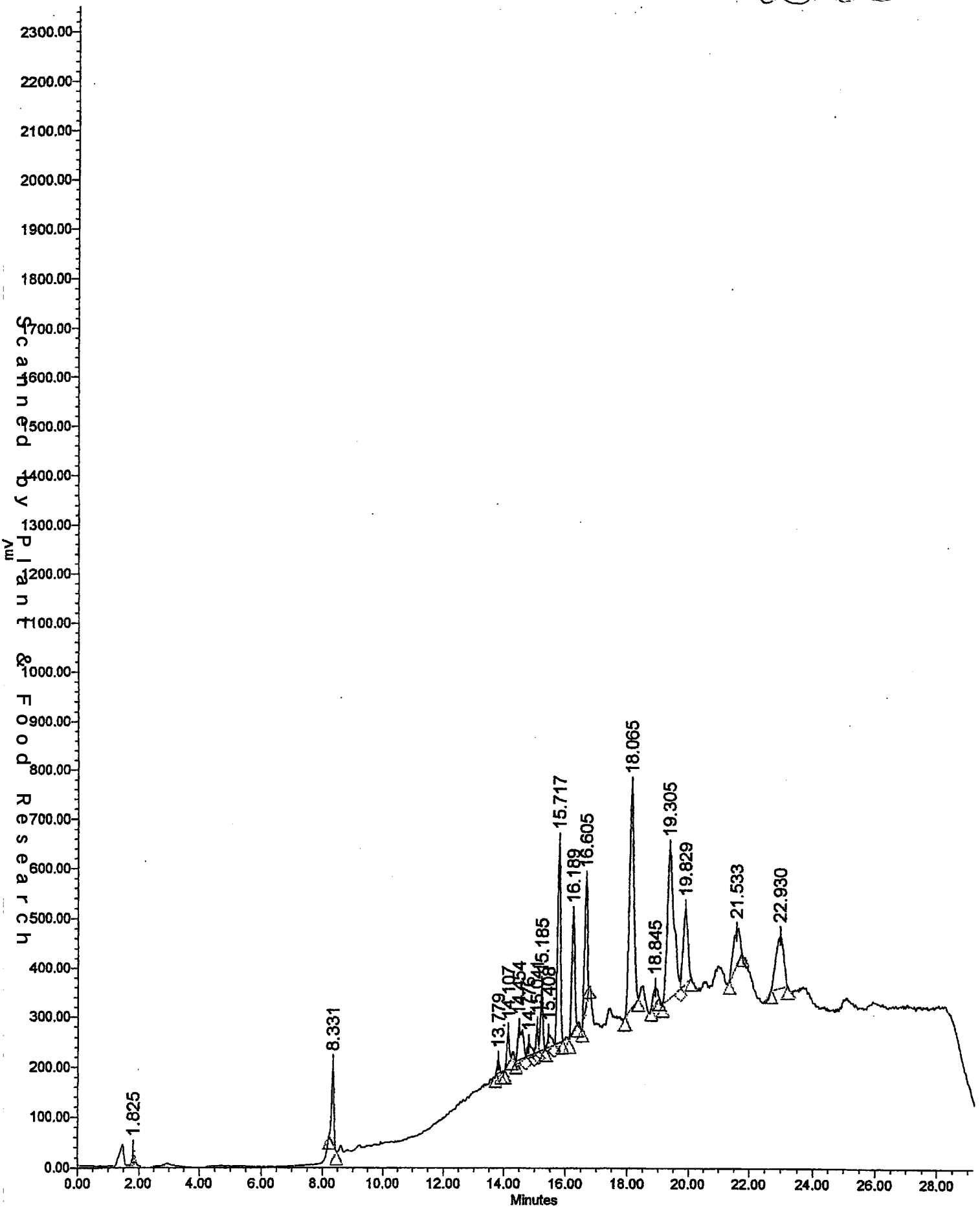


Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.549	390707	56786		
2		1.799	254296	44569		
3		8.334	234145	48027		
4		13.774	252813	55054		
5		14.101	1376447	231710		
6		14.259	609641	93749		
7		14.440	4305946	529979		
8		14.754	1603690	173506		
9		15.038	788822	125474		
10		15.184	1211382	258336		

	Name	RT	Area	Height	Amount	Units
11		15.419	478517	55774		
12		15.719	2763925	379701		
13		16.195	2209987	300072		
14		16.359	339510	59518		
15		16.602	21000650	2433434		
16		17.395	649100	87242		
17		18.078	20367598	2045374		
18		18.442	1547655	135568		
19		18.868	2047735	151119		
20		19.309	22521014	1698962		
21		19.849	10702968	713320		
22		20.495	254425	34926		
23		20.931	2129041	113868		
24		21.473	20448945	799186		
25		22.800	3236031	273382		
26		24.778	45961	12764		
27		25.072	510252	51510		
28		25.100	108099	38529		

7lb Pacific Purple Whale



Project Name JOCELYN
User Name System
Software Version 3.20

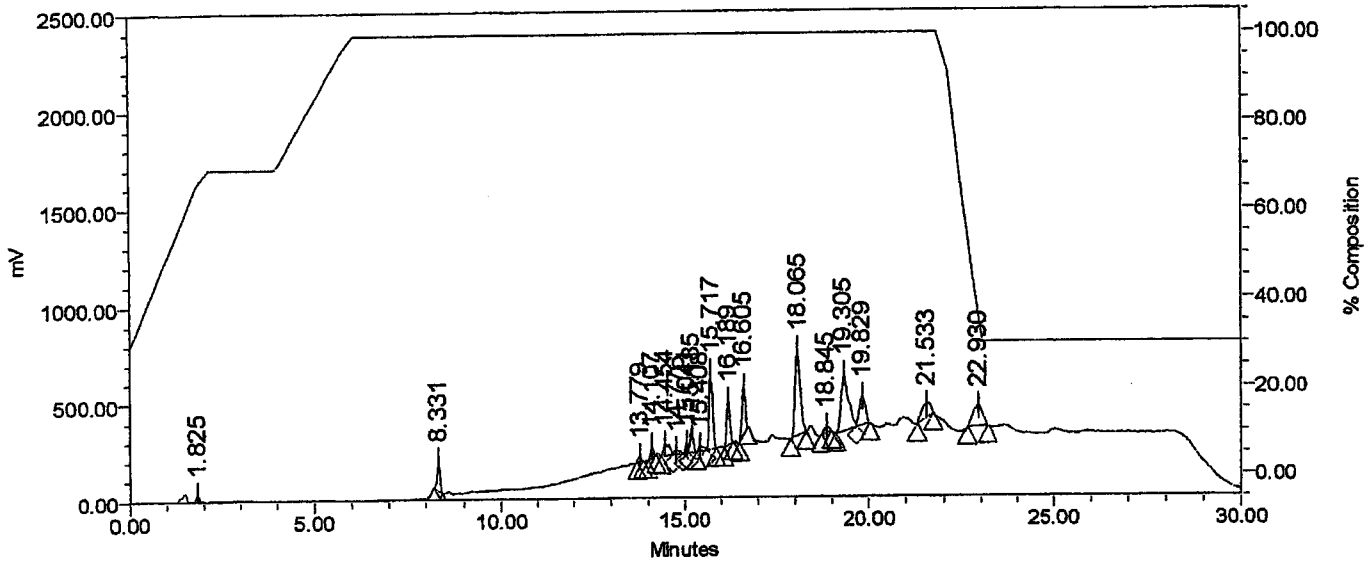
Report Method Name saponin
Current Date 7/3/03

Sample Information

SampleName	Asparagus 9b
Vial	3
Injection	1
Injection Volume	50.00 ul
Channel	SATIN
Run Time	30.0 Minutes

Sample Type	Unknown
Date Acquired	5/8/03 2:54:35 PM
Acq Method Set	Saponin elsd
Processing Method	saponin
Date Processed	7/3/03 4:29:10 PM

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.825	124386	26691		
2		8.331	829544	160585		
3		13.779	130616	25939		
4		14.107	345848	63385		
5		14.454	722451	65258		
6		14.776	230692	24623		
7		15.041	250142	52548		
8		15.185	684095	132169		
9		15.408	243155	28032		
10		15.717	2763254	402833		

	Name	RT	Area	Height	Amount	Units
11		16.189	1519691	239214		
12		16.605	1555117	257561		
13		18.065	4413923	450393		
14		18.845	305360	32718		
15		19.305	4331186	303804		
16		19.829	1724139	150449		
17		21.533	1122349	69737		
18		22.930	1800924	106950		

Joint with base

Project Name JOCELYN
User Name System
Software Version 3.20

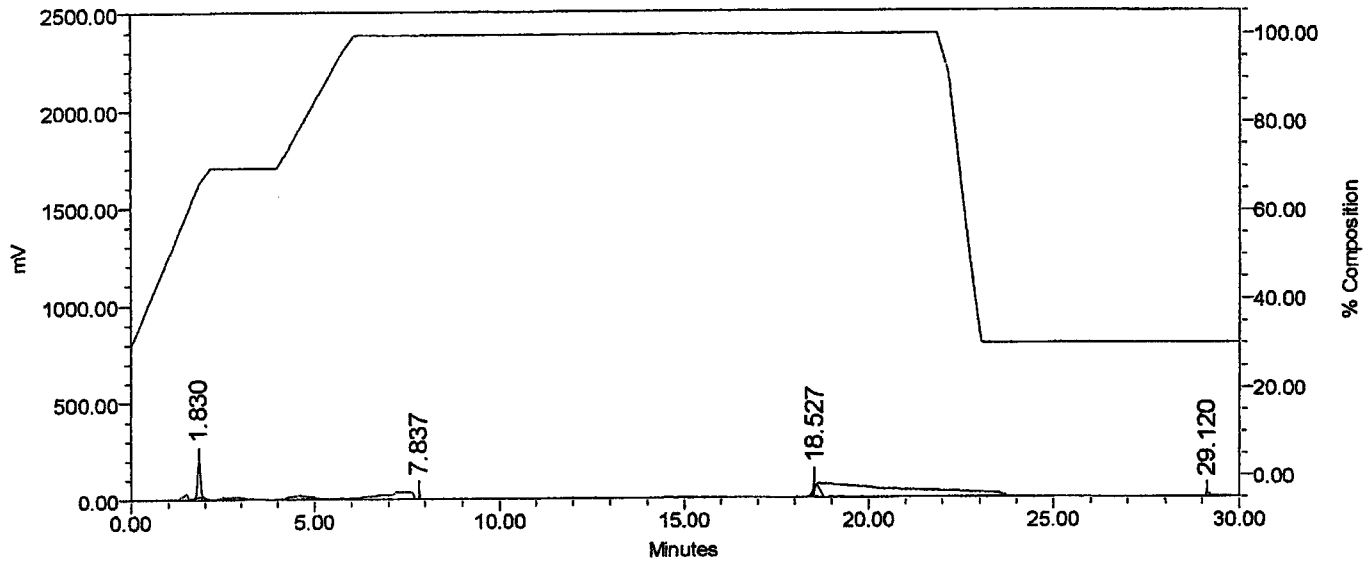
Report Method Name saponin
Current Date 7/3/03

Sample Information

SampleName	Asparagus 10b
Vial	2
Injection	1
Injection Volume	50.00 ul
Channel	SATIN
Run Time	30.0 Minutes

Sample Type	Unknown
Date Acquired	5/8/03 3:26:19 PM
Acq Method Set	Saponin elsd
Processing Method	saponin
Date Processed	7/3/03 4:28:06 PM

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.830	1065931	193834		
2		7.837	74002	28864		
3		18.527	186194	62493		
4		29.120	128005	18416		