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Determination of Wine Aroma Compounds from Simple Extracts using Automated Large Volume Injection with PTV Solvent Splitting

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INTRODUCTION

A combination of sensory testing and analysis of individual chemical species is now regarded as necessary, both for evaluation of wine character and quality as well as for a definite determination of off-flavours. In this regard advances in methodology for rapid determination of aroma compounds is an important develeopmental area.

The aroma of wine is determined through the combined effects of several hundred chemically different compounds where the concentration of a single compound can vary between 10^{-4} and 10^{-11} g/l [1-6]. To enrich aroma compounds as a basis for quantification liquid/liquid extraction using different solvent/solvent-mixtures (e.g. pentane/ether, pentane/dichloromethane) is suitable [3-6]. Due to its properties (purity, low boiling point, low solubility for ethanol, inflammability) trichlorofluoromethane has proven to be a suitable solvent for an artefact-free and comprehensive extraction of aroma compounds from aqueous and aqueous/ethanolic matrices [3-11].

Such extractions can give complex aroma concentrates containing several hundred single compounds. These can be used to characterize wines of different grape varieties [2-6, 9-11, 18-21], but in many cases only the determination of the main wine aroma compounds (e.g. fermentation aroma and main compounds of grape variety aroma) is of interest.

These main compounds of the fermentation aroma (e.g. higher alcohols) can partially be quantified through direct injection on packed, micro-packed or capillary columns. Due to a wine matrix with many compounds of low volatility and non-volatiles (sugars, acids, glycerine, phenols, dyes, etc.) the inlet system can be contaminated and separation and quantification can be compromised through the high concentration of compounds like water, ethanol, glycerine, etc.

Although the problem of injecting compounds of low volatility and non-volatiles can be solved using headspace techniques detectability is only sufficient for a few compounds [22]. An additional possibility to separte non-volatiles and simultaneously enrich the volatiles is offered by distillation. From 100 ml wine 25 ml of distillate is produced, 1 µl is directly injected and approx. 20 main compounds (alcohols,

esters) quantified [23, 24]. The detection limits for each ester are at approx. 0.01 mg/100 ml.
In this paper a simple and fast method for enrichment and quantification of volatile compounds in wine using a solvent venting technique is described. 10 ml of wine and only 100 µl of extractant are necessary. The aroma extracts can directly be used for GC/MS analysis, without any further sample preparation.

EXPERIMENTAL

Sample preparation. A vial with tapered bottom (total volume 12 to 15 ml) and ground joint is filled with 10 mls of wine, 10 µl of a 0.1% standard solution (100 µl 2,6-dimethyl-5-heptene-2-ol in 100 ml ethanol) and 100 µl 1,1,2-trichlorotrifluoroethane (Kaltron). Instead of 2,6-dimethyl-5-heptene-2-ol also 2-ethylhexanol or decanol-3 can be used (depending on the separation efficiency of the capillary column). For better extractability (alcohols, acids) und for reduction of emulsion formation 2 g NaCl or 4.2 g $(NH_4)_2SO_4$ may be added to the wine. The vial is then closed and mechanically shaken for 15 minutes. After that the Kaltron phase is separated and found at the bottom of the vial. If an emulsion is formed a short period of centrifugation (5 min at 2500-3000 U/min) will separate the phases. The solvent phase can be used without any further pretreatment for large volume injection with solvent venting.

Standard preparation. For preparation of standard solutions as basis for quantification of single compounds (standard addition) a defined amount of a parent solution (10 mg of each compound per ml ethanol) is added to 10 mls of wine. For example 10 µg/l of this parent solution added to 10 mls of wine will increase the concentration of each single compound by 100 mg/l.

Instrumentation. The applied system consists of a Multi Purpose Sampler (Gerstel GmbH, Mülheim an der Ruhr, Germany, **Figure 1**), operated in large volume injection-mode and equipped with a 100 µl

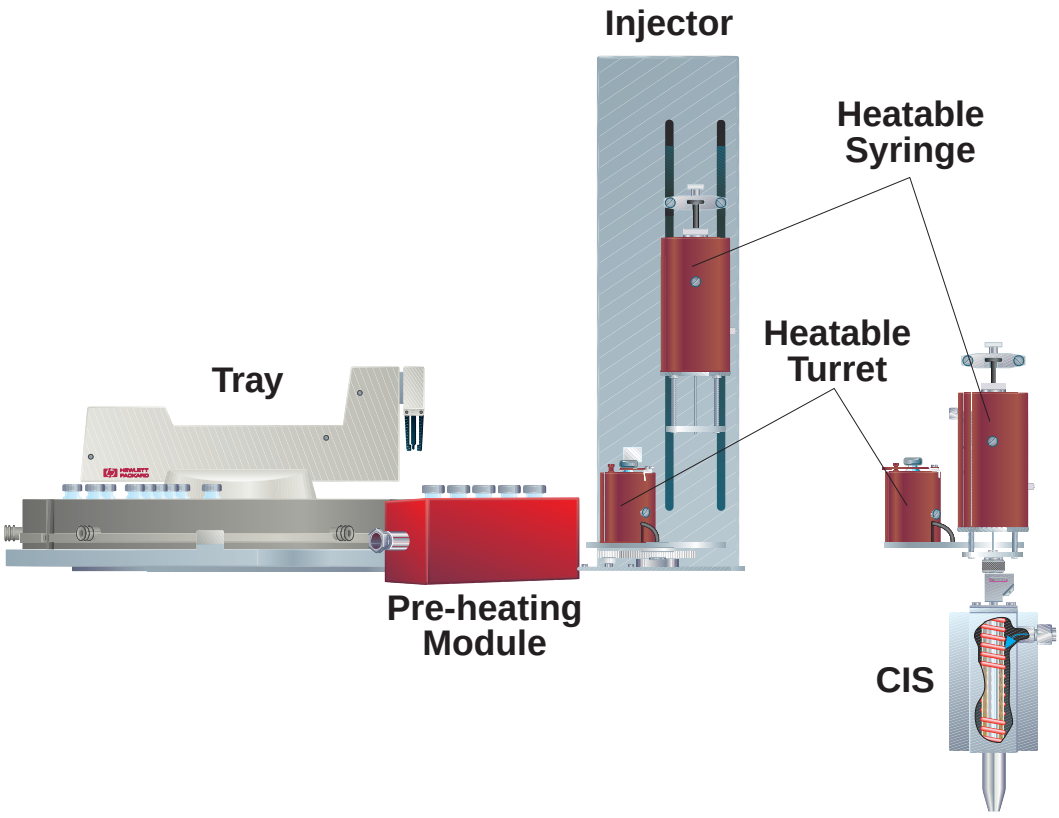

Figure 1. *Gerstel Multi Purpose Sampler, here used as Large Volume Injector. Heated zones can be used optionally or for headspace-mode.*

syringe, a HP 7673 tray for 100 2ml standard vials (Hewlett-Packard, Waldbronn, Germany), a temperature controlled cooled injection system CIS (Gerstel GmbH, Mülheim an der Ruhr, Germany, **Figure 2**) used as interface, cold trap and injection system for the subsequently following GC-MSD combination (HP 5890/5972, Hewlett-Packard, Waldbronn, Germany).

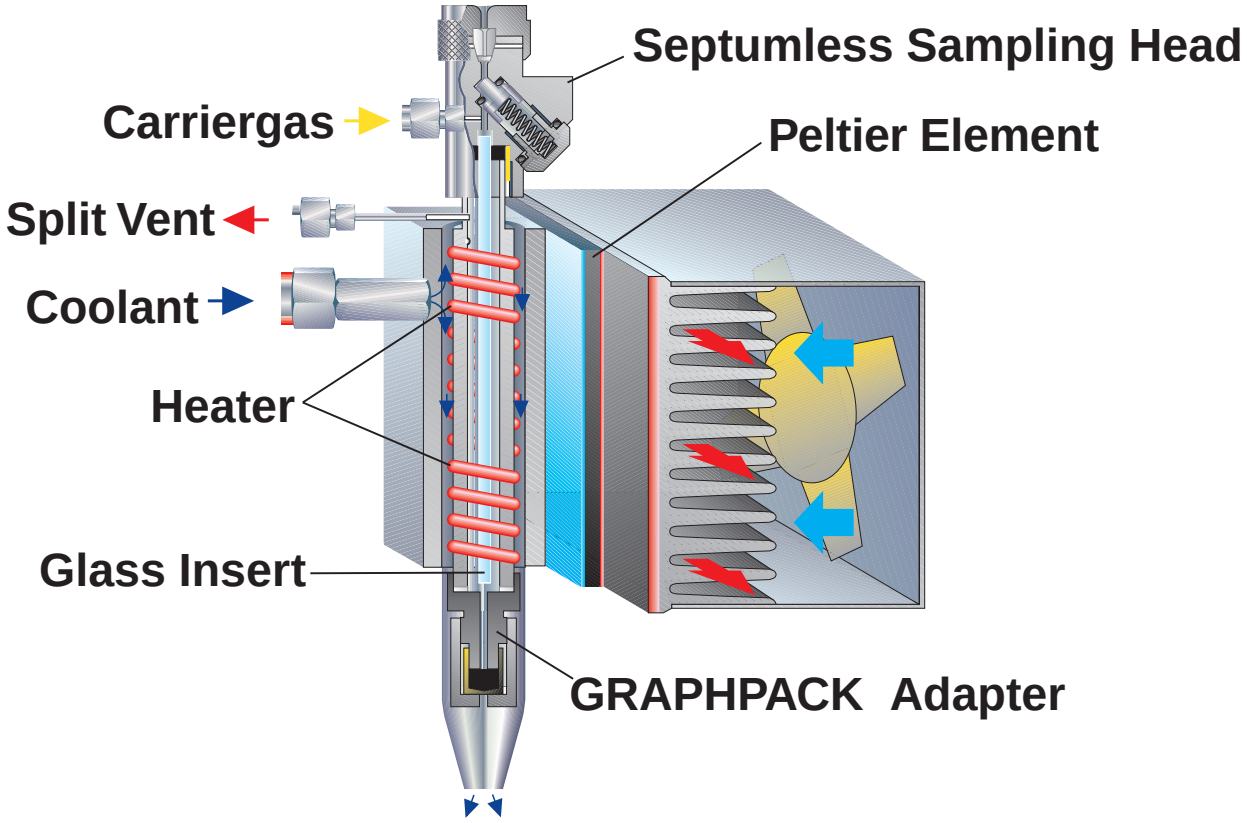

Figure 2. *Gerstel Cooled Injection System CIS 3.*

Large Volume Injection. This is a rapidly developing technique which can be fully automated, and promises to substantially extend the applicability of capillary gas chromatography [12-17, 25]. The Multi Purpose Sampler fills the syringe with sample, where the depth of injection is controlled for both the position in the vial and in the CIS. With the split vent open and the CIS cooled down to subambient temperatures the sample is injected with programmed speed into the glass insert. The split flow through the liner at these temperatures preferentially removes the solvent as a vapour while leaving the solutes of interest in the liner. After the solvent has been vented, the split is closed and the CIS is ramped to the desired temperature for splitless transfer of the analytes to the capillary column.

Analysis conditions.

Injector:	25 µl injection volume, at 20 µl/min	
Columns:	60 m HP Innowax (Hewlett Packard),	d_i=0.25 mm, d_f=0.25 µm
Pneumatics:	He, p_i = 24 psi,	purge flow = 80 ml/min,
		purge time = 1 min, splitless time = 1 min
Temperatures:	CIS:	10°C (1.5 min) to 250°C (10 min), with 10°C/s
	Oven:	40°C (1 min) to 240°C (20 min), with 2°C/min
	MSD:	280°C
Detector:	MSD, Scan 25 - 300 amu	

RESULTS AND DISCUSSION

Figure 3 shows the total ion chromatogram of a large volume injection of a Kaltron extract obtained from a wine of the Morio-Muskat grape. Many compounds from wine aroma can be analysed with this simple and fast and at the same time gentle and therefore artefact free sample preparation/enrichment method (up to more than 100 µl with solvent venting technique). The GC system is not contaminated with low volatility or non-volatile compounds (glycerine, sugar, dicarbonacids, dyes, etc.), unavoidable when injecting the wine directly. In addition the capillary column is protected from high amounts of water.

In addition to fermentation aroma compounds like 3-methyl-1-butanol, 2-phenyl ethanol, caprylic acid, capric acid, fatty acid methyl esters, diethyl succinate, numerous additional aroma compounds characteristic for a grape type (e.g. hexanol, linalool, α-terpineol, geraniol, linalool oxide) can also be analysed. Therefore this fast and simple enrichment method (using only 5-10 ml of sample and 50-100 µl of solvent) can be utilized as a basis not only for quality control analysis of the fermentation byproducts, but also for characterization of grape varieties through determination of typical varietal aroma compounds.

List of compounds (**Figure 3**)

1	2-Methyl-1-Butanol	18	Ethyl Caprate	34	Diethyl Malonate
2	3-Methyl-1-Butanol	19	Diethyl Succinate	35	Caprylic Acid
3	Ethyl Caproate	20	α-Terpineole	36	Ethyl Cinnamate
4	Ethyl Lactate	21	Terpenediol-I	37	Butyric Acid-i-Butyl Ester
5	Hexanol	22	Methionol	38	Capric Acid
6	3-Hexene-1-ol	23	Citronellol	39	Phenyl Acetic Acid-i-Butyl Ester
7	2-Hexene-1-ol	24	cis-p-Linalool Oxide		
8	Ethyl Caprylate	25	trans-p-Linalool Oxide	40	trans-Geranylic Acid
9	cis-(f)-Linalool Oxide	26	Benzene Acetic Acid Ethyl Ester	41	Myristic Acid
10	Octene-1-ol-3				
11	trans-(f)-Linalool Oxide	27	Nerol		
12	3-OH-Ethyl Butyrate	28	Phenyl Ethyl Acetate		
13	Linalool	29	Ethyl Laurate		
14	Acetoine	30	Geraniol		
15	i-Butyric Acid	31	Caproic Acid		
16	Hotrienol	32	Benzene Methanol		
17	Butyric Acid	33	2-Phenyl Ethanol		

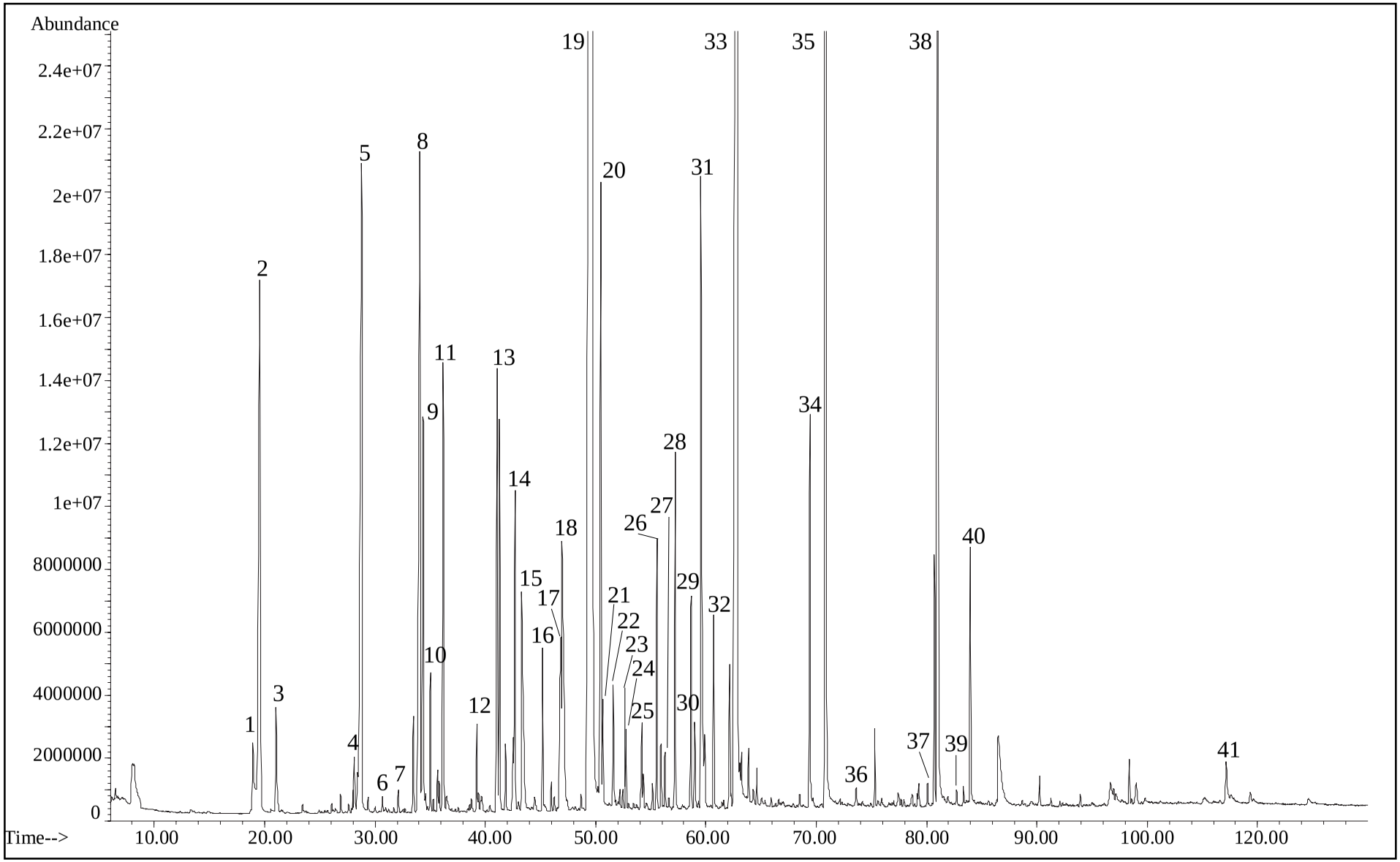

Figure 3. *Total ion chromatogram of a large volume injection of a Kaltron extract obtained from a wine of the Morio-Muskat grape ("Fingerprint"-chromatogram).*

Grape varieties can be differentiated through comparison of the aroma patterns ("fingerprints"). An example is given in **Figure 4**, comparing the "fingerprint" chromatograms of the Morio-Muskat and the Silvaner grape. Morio-Muskat for example contains higher amounts of monoterpene compounds (linalool, α-terpineol, geraniol) as the neutral grape type of Silvaner. With the help of multiple discrimant analysis techniques these compounds can be used to identify different grapes [5,6,10,12-15].

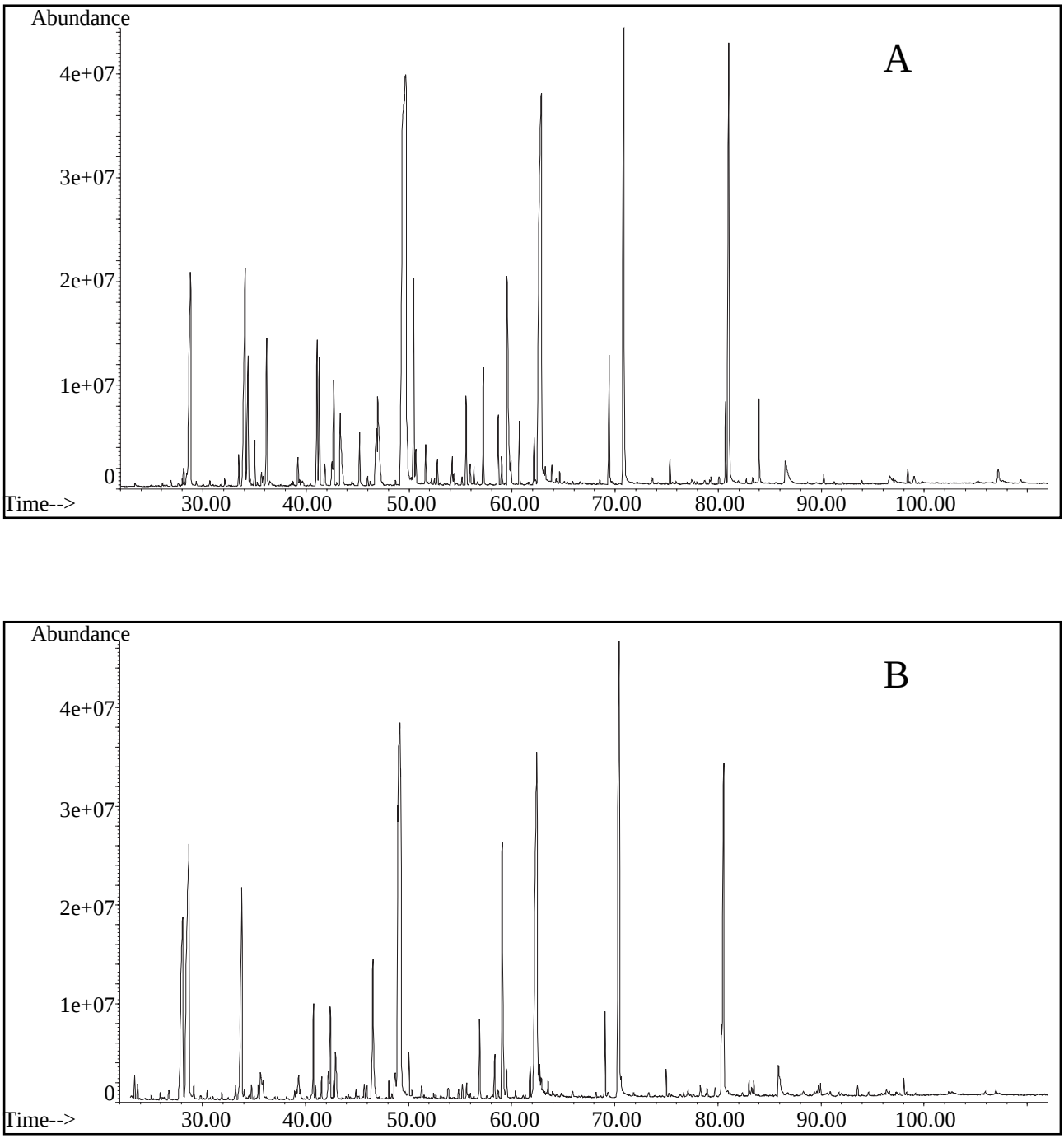

Figure 4. *Comparison of "fingerprint" (total ion) chromatograms of large volume injections of Kaltron extracts obtained from wines of the Morio-Muskat grape (A) and the Silvaner grape (B).*

Conclusion

Large volume injection of simple wine extracts is an attractive alternative to normal injection of classical extracts for determination of both fermentation and varietal aroma compounds. Major advantages for the laboratory are replacement of expensive, continuous extraction procedures, but with the detection limits and sensitivity regained by large volume injection of simple extracts. A further significant advantage is that every function of the large volume injection operation (injection volume, injection speed, injector temperature, etc.) can be automated, and multiple samples injected using existing Hewlett-Packard autosampler hardware.

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