Separation of 17 DNPH derivatised carbonyles using the Chromaster 600 bar HPLC system with UV detector and semi-micro flow cell

Carbonyl compounds are often found in urban atmospheres and can give rise to photo-chemical smog and reactions leading to ozone formation. The main source of these compounds includes products of incomplete hydrocarbon combustion produced by internal combustion engines. Insulating materials used in buildings and furniture can also be a source of such compounds in the atmosphere.

Large quantities of carbonyl compounds are used worldwide as raw materials and intermediates in the chemical and plastics manufacturing industries. These compounds are skin, eye and respiratory system irritants, many are also suspected of being carcinogenic. Owing to widespread, high volume industrial use of such compounds, they are present in workplace air spaces and industrial waste materials. As a result of this they are also present in municipal waste burial sites, which can contaminate groundwater that may eventually be used for drinking.

It is therefore important to be able to quickly qualify and quantify these compounds in a reliable and reproducible way, however, a number of carbonyl compounds that are often present in air samples have no chromophores. For low molecular weight aldehydes and ketones in air and water, HPLC with an in-situ derivatisation of carbonyls using 2,4-dinitrophenyl-hydrazine (DNPH) (Figure 1) is a reliable way of quantifying the amount of these carbonyls present.

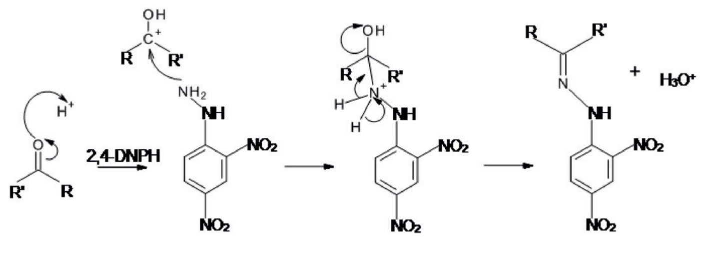
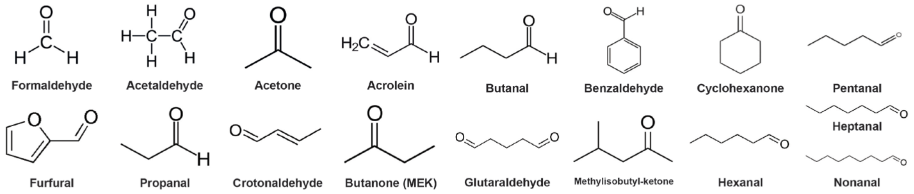


Figure 1. Derivitisation reaction of carbonyl group with 2,4-DNPH.



This application note gives an HPLC method for the separation and detection of carbonyl compounds in aqueous samples. Extracts are first derivatised with 2,4 dinitrophenylhydrazine (DNPH). After derivatisation, the carbonyl compounds are extracted and injected onto a Merck Hibar® HR 100-2.1 mm Purosher® STAR RP-18e, 2 µm column and separated using gradient elution. The separated compounds are then quantified by UV detection at a set wavelength of at 360 nm. These analytical procedures are consistent with U.S. EPA Method 554, pertaining to drinking water samples and U.S. EPA Method 8315, pertaining to aqueous and solid waste, soil, stack gas and indoor air samples. In this method the VWR Hitachi Chromaster is used in a mixerless pump formation, meaning that the system delay volume is kept to a minimum. The high performance Chromaster proportioning valve is used in a high frequency mixing mode (HFM) instead of a mixer. This use of Hitachi’s expertise in high performance electronics has greatly improved the design of low pressure gradient (LPG) accessories, meaning that in a number of applications a high pressure gradient binary system is not necessary. This makes use of the flexibility of a LPG quaternary system where by tetra hydrofuran is used as a third component of the mobile phase. The THF aids the separation of the more polar components by forming an intermediate complex to slightly reduce the polarity of these derivitised compounds.

The cut method of sample injection in the 5260 autosampler gives the Chromaster a highly reproducible injection performance. Care should be taken to make sure the correct lead and rear volume is programmed in the method.

Chromatographic conditions and ordering numbers

5160 Pump 600	No mixer, Gradient Mode: HFM **Cat. No. 903-0554**
Low pressure gradient unit for 5160	**Cat. No. 903-0562**
Degasser	6-channel degassing unit **Cat. No. 903-0503**
5260 Autosampler	Cut-Method, lead vol.: 5 µl, rear vol.: 30 µl; syringe: 175 µl **Cat. No. 903-0556**

Eluents		**A: Water**	**B: Acetonitrile**	**C: Tetrahydrofuran**
Gradient	**Time**	**A%**	**B%**	**C%**
	0	64	20	16
	3	64	20	16
	15	45	55	0
	22	20	80	0
	25	20	80	0
	25.1	64	20	16
	30	34	20	16

Chemicals

Description	Cat. No.
Acetonitrile gradient grade	1.00030.1000
Tetrahydrofurane for HPLC	1.08101.1000

In Figure 2 it can be seen that the 17 components from a standard solution are clearly separated. In Figure 3 an overlay of two standard chromatograms (500 ng/ml and 100 ng/ml) and a real air sample shows the efficacy of this method.

There is a lack of good baseline separation between some components such as glutaraldehyde (peak 13) and methylisobutylketone (peak 14) however, it is certainly a usable and most importantly, reproducible method.

The Chromaster, as a 600 bar low pressure gradient HPLC system, is an ideal system to produce a chromatogram with the required resolution for quantification at nanogram levels using a UHPLC column with a 2 µm particle size. The Hitachi low pressure gradient pump set up gives an excellent price/performance ratio with all the flexibility that a quaternary LPG has to offer compared to a high pressure binary system.

The renowned robustness of the Chromaster can help reduce system down time and decrease maintenance costs. For a demonstration or further information, please email chromjournal@eu.vwr.com

Bypass capillary between pump and autosampler

5310 Oven	**Cat. No. 903-0520**
5410 UV detector	**Cat. No. 903-0524 -** Semi-micro retrofit kit including semi-micro cell (5 mm, 3.2 µl) **Cat. No. 903-0589**
Organiser cabinet for solvent bottles	**Cat. No. 903-0537**
IF control board	**Cat. No. 903-0545**

Flow rate/run time	0.4 ml/min - 30 min including re-equilibration time
Pressure	520 bar
WL	360 nm, resp.time: 1.0 s, sampling period: 400 ms, A/Z before injection
Oven temperature	40 °C
Injection volume	6 µl
Column	Merck Hibar® HR 100-2.1 mm Purosher® STAR RP-18e, 2 µm, **Cat. No. 1.50648.0001**

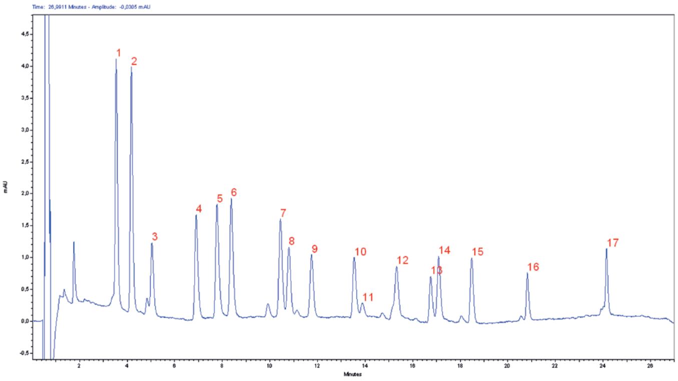


Figure 2. 6 µl of a DNPH derivatised standard mix, 100 ng/ml each.

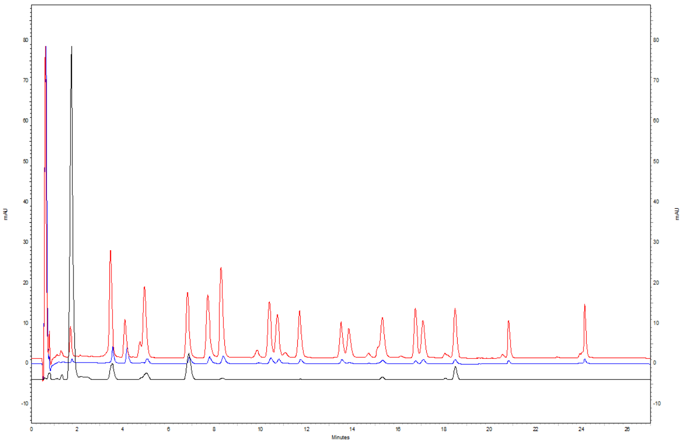


Figure 3. Overlay of a real DNPH derivatised air sample with the two standard mix.