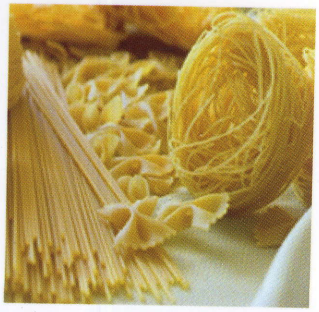

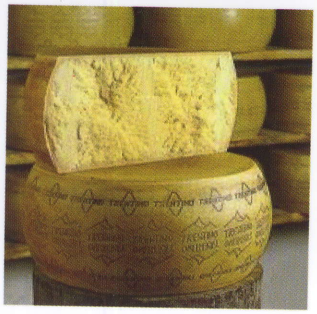

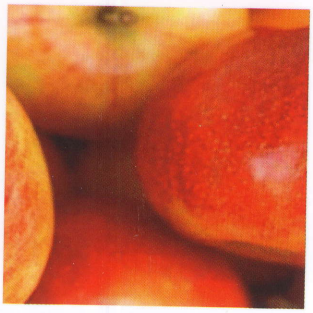

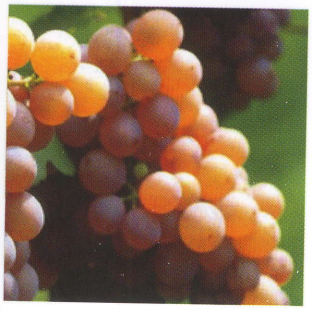

Divisione di
Spettrometria di Massa

FONDAZIONE
EDMUND
MACH

BOOK OF ABSTRACTS

3rd MS Food Day

2013 October 9-11
Trento - Italy

Organised by:
Fondazione Edmund Mach
Italian Chemical Society

EuCheMS

3rd MS FOOD DAY 2013

October 9-11, 2013
University of Trento

BOOK OF ABSTRACTS

Franco Biasioli
Editor

Fondazione Edmund Mach - 2013

P.34 - Phthalates determination in wine and spirits using GC-MS and LC-MS/MS

Luca Raveane, Loris Tonidandel, Sergio Moser, Debora Trainotti, Tiziana Nardin, Roberto Larcher*

Fondazione Edmund Mach (FEM), via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
* *roberto.larcher@fmach.it*

Summary: Phthalates, widely studied in the last years as insidious and ubiquitous contaminants of industrially processed foods, have recently been reported also in some wines and spirits. This work presents and compares the performances of two novel analytical approaches for the simultaneous determination of seventeen phthalates (i.e. DMP, DEP, DiBP, DBP, DMEP, BMPP, DEEP, DPP, DHXP, BBP, DBEP, DCHP, DEHP, DPhP, DNOP, DNP, DiNP; EN ISO 1043-3:1999 D) using mass spectrometry in alcoholic beverages. GC-MS analysis requires a preliminary time-consuming sample extraction, the use of perfectly cleaned glassware, highly-purified extraction solvents, and the concentration of the final organic extract. LC-MS/MS method is faster (no LLE and concentration steps) and more sensitive, even if it requires ultra-purified eluents, or the adoption of an on-line device for the background removal. Both approaches proved to be rugged and useful for routine and high-throughput analysis. DEHP, DiBP, DBP, DMEP and DiNP were present in more than 70% of spirits, while DBP and DNOP were quantifiable in 60% of wines.

Keywords: Beverages; Mass Spectrometry; Plasticizers

Introduction

Phthalates are a wide group of esters of o-phthalic acid. High molecular weight phthalates (e.g. DEHP and DiNP) are mainly used as plasticizers to soften PVC products; lower molecular weight phthalates are used as solvents for colour and scent in various personal care products [1]. Their penetration into the environment and food occurs because they are not chemically bond to the plastic polymers. Phthalates have been classified as reproductive and developmental toxicants on the basis of their ability to interfere with the endocrine system; these compounds and their metabolites can have adverse effects on reproductive system [2]. The aim of this work is the validation and the comparison of two innovative approaches, using gas chromatography and liquid chromatography both coupled with mass spectrometer, for a rapid, accurate and high throughput determination of phthalates in alcoholic matrices.

Experimental

Forty commercial and spiked alcoholic beverages (wines, distillates, liquors) were analyzed both in GC-MS and LC-MS/MS. For the GC analysis, 25 mL of sample was extracted with 3 x 5 mL of dichloromethane (DCM) in a separating funnel. The 3 fractions, put together and anidrified with sodium sulphate anhydrous, were concentrated under nitrogen flow to 1.0 mL and analyzed using an Autosystem XL (Perkin-Elmer; split injection 1:20, DB-5ms 30m, 0.25 mm i.d., 250µm) coupled with a Turbomass Gold (Perkin-Elmer; EI, 70eV, 50µA). The sample preparation was particularly time-consuming because glassware and sodium sulphate needed to be carefully washed, at least tree times, with acetone and n-hexane. The calibration solutions (from 10 to 5000 µg/kg) were prepared in DCM starting from a multi-standard stock solution containing 1.0 g/L of each analyte and using d4-DEP (0.5 mg/kg; deuterations only on the aromatic ring) as internal standard. The sample for the LC analysis was only 40 times diluted (water/methanol, 1:1), filtered (0.22 µm) and injected (20 µL) into a UPLC Acquity (Waters; column BEH, C18, 1.7 µm, 2.1 x 100 mm;) and analyzed by Xevo TQ (Waters; ESI+, Cone

voltage = 20V). An accurate quantitation requires phthalates-free eluents to prevent interferences in the chromatographic separation, but a specific background-removal devices (Isolator Column; Waters) or also a common UPLC BEH C18 column (Waters;2.1x50 mm, 1.7 µm), both inserted in the fluidic system before the injector valve, proved to be very successful in delaying the chromatographic exit of the phthalates present in the usual commercial solvents. The calibration solutions (10.0 - 1000 µg/kg) were prepared in methanol from a multi-standard stock solution containing the analytes at 2.5 mg/kg and d4-DEP at 50.0 µg/kg.

Results

Table 1 presents the details of mass methods. Both GC-MS and LC-MS/MS provide very sensitive and linear (always R2>0.99) calibration curves. The Limit of detection (as 3σ) varied for GC-MS between 0.02 to 3.0 µg/kg, while for LC-MS/MS between 0.01 and 0.50 µg/kg. Recoveries on spiked samples were quantitative (around 100%) for all the compounds using LC-MS/MS and between 70-120% using GC-MS. Is noteworthy in spirits the ubiquitous presence of DEHP (100% samples) with median content at 350 µg/kg and a maximum at 3900 µg/kg, while DiBP, DBP, DMEP and DiNP were commonly present (70%). Median values were respectively of 20, 35, 40 and 40 µg/kg and maximum values of 4300, 270, 420 and 3400 µg/kg. Other phthalates quantified with lower occurrence (less 50% samples) were DMP, DEP, DNOP and DNP. 8 phthalates were never quantifiable. In wines only DBP and DNOP were quantified (60% samples) with median values of 30 and 60 µg/kg and maximum values of 50 and 200 µg/kg, respectively. Detectable but not quantifiable amounts of DEP, DiBP, DEHP and DPhP were sometimes founded.

Tab 1. Mass parameters for GC-MS and LC-MS/MS. In square brackets are reported the collision energies (CE) in Volts. LOQ = Limit of Quantification.

Abbr.	Common Name	GC-MS		LC-MS/MS	
		M$^+$, quantifier, and qualifiers ions	LOQ (µg/kg)	Transitions: quantifier [CE], qualifier [CE]	LOQ (µg/kg)
DMP	Dimethyl phthalate	194, 163, 77	10	195 > 163 [5], 195 > 77 [30]	0.6
DEP	Diethyl phthalate	222, 149, 177	2.5	223 > 149 [20], 223 > 177 [10]	1.6
DiBP	Diisobutyl phthalate	278, 149, 223	0.06	279 > 149 [5], 279 > 205 [5]	0.5
DBP	Di-n-butyl phthalate	278, 149, 223	4.0	279 > 149 [5], 279 > 205 [5]	0.5
DMEP	Bis(2-methoxyethyl) phthalate	282, 59, 149	10	283 > 207 [5], 283 > 59 [10]	0.03
BMPP	Bis(4-methyl-2-pentyl) phthalate	334, 149, 85	10	335 > 251 [10], 335 > 85 [15]	0.1
DEEP	Bis(2-ethoxyethyl) phthalate	310, 45, 72, 149	10	311 > 73 [10], 311 > 149 [25]	0.4
DPP	Dipentyl phthalate	306, 149, 43	10	307 > 219 [10], 307 > 149 [5]	0.08
DHXP	Di-n-hexyl phthalate	334, 149, 251	10	335 > 233 [10], 335 > 121 [25]	0.8
BBP	Benzyl butyl phthalate	312, 149, 91	10	313 > 91 [15], 313 > 149 [15]	0.1
DBEP	Bis(2-n-butoxyethyl)phthalate	366, 149, 57, 110	10	367 > 249 [5], 367 > 101 [10]	0.3
DCHP	Dicyclohexyl phthalate	330, 149, 167	10	331 > 149 [10], 331 > 167 [15]	0.8
DEHP	Bis(2-ethylhexyl) phthalate	390, 149, 167	0.07	391 > 167 [15], 391 > 279 [10]	1.3
DPhP	Diphenyl phthalate	318, 225, 77	10	319 > 225 [5], 319 > 77 [20]	0.1
DNOP	Di-n-octyl phthalate	390, 149, 279	2.5	391 > 121 [40], 391 > 261 [10]	1.6
DiNP	Diisononyl phthalate	418, 149, 293	10	419 > 149 [10], 419 > 71 [15]	1.6
DNP	Di-n-nonyl phthalate	418, 149, 293	10	419 > 149 [10], 419 > 71 [15]	1.6

Conclusion

Phthalates, a big challenge for food analysts, can be effectively quantified in alcoholic beverages using both GC-MS and LC-MS/MS. The liquid chromatographic method, allowing to avoid time-consuming sample and glassware preparation, is faster (15 min vs. 45 min in GC), but also more sensitive. Nevertheless, matrix effects during the ionization steps can be sometimes observed and a sample dilution (at least 1:30) is generally required. In-matrix calibration curves can not be easily prepared for the ubiquitous presence of phthalate traces in commercial samples. LODs of both the approaches are in accordance with scientific literature [3] and consistent with the Maximum Residue Levels defined for the alcoholic beverages trade by some extra-EU custom authorities. For routine LC-MS/MS analysis the use of an

'accumulating/exit-delaying' column can be profitably used to improve the pureness of common eluents.

References

[1] Y. Guo, K. Kannan; Analytical Bioanalytical Chemistry; 404 (2012) pp. 2539-2554.
[2] C. Xu-Liang; Comprehensive Reviews in Food Science and Food Safety; 9 (2010) pp. 21-43.
[3] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. Mastrocola, A. Cicchelli; Food Chemistry; 111 (2008) pp. 771-777.

ISBN 978-88-7843-035-8

3rd MS Food Day

Trento – Italy

2013 October 9-11