Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Critical evaluation of several techniques for the analysis of phthalates and terephthalates: Application to liquids used in electronic cigarettes

Serban C. Moldoveanu*, Ravikiran Yerabolu

R.J. Reynolds Tobacco Co., 950 Reynolds Blvd., Winston-Salem, NC, 27105, United States

ARTICLE INFO

Article history:
Received 29 September 2017
Received in revised form 20 January 2018
Accepted 1 February 2018
Available online xxx

Keywords:
Phthalates
Terephthalates
GC/MS/MS
LC/MS/MS
e-Liquids

ABSTRACT

This study describes several original methods that were developed with the goal of measuring phthalates and terephthalates. These methods include gas chromatography/mass spectrometry (GC/MS), GC/MS/MS, liquid chromatography with UV detection (LC/UV), LC/MS, and LC/MS/MS. The study compares the advantages and disadvantages of these methods and their applicability to measuring phthalates and terephthalates in the liquids used in electronic cigarettes (e-liquids). The analytes evaluated include eight phthalates and two terephthalates. The phthalates were diethyl, dibutyl, benzyl butyl, diphenyl, bis(2-ethylhexyl), di-*n*-octyl, diisononyl and diisodecyl. The terephthalates were dimethyl and bis(2-ethylhexyl). Intentionally, no cleanup or concentration step were used in the methods. The methods used two chromatographic standards, dimethyl phthalate-3,4,5,6-d_4, and di-(2-ethylhexyl) phthalate-3,4,5,6-d_4. All techniques were validated for selectivity/specificity, precision, sensitivity (evaluation of LOD and LOQ), as well as for repeatability and matrix interference. The GC methods were not adequate for the analysis of diphenyl, diisononyl, and diisodecyl phthalates which were not volatile enough to be seen in the conditions used for the GC separation. Also, alcohols should not be used as solvents for the injection of the sample in the GC system to avoid transesterification in the hot injection port. The single quadrupole MS detection in GC offers sensitivities around 1 μg/mL in the e-liquid and was not sensitive enough for the analysis of trace phthalates and terephthalates. Compared to all evaluated methods, the MS/MS detection in GC offered the best sensitivity (below 10 ng/mL in the e-liquid). The LC is adequate for the separation of all the evaluated analytes. However, the UV detection in LC does not offer good sensitivity compared to all the other techniques. The MS detection in LC provides poor sensitivity for terephthalates, but better than the UV for the rest of the analytes. The MS/MS detection for LC offers slightly better sensitivity than the MS detection, but both LC/MS and LC/MS/MS were only able to measure levels above about 100 ng/mL of analytes in the e-liquid. A group of 39 e-liquids were analyzed by three of the evaluated procedures. Benzyl butyl phthalate, bis(2-ethylhexyl) terephthalate, and di-*n*-octyl phthalate were not detected in the e-liquids. Some of the other evaluated phthalates were present at trace levels in certain e-liquids while most e-liquids did not contain phthalates at detectable levels.

1. Introduction

The esters of 1,2-benzenedicarboxylic acid (phthalates) are frequently utilized as plasticizers for polymers, as well as additives in paints, glues, cosmetics, etc. The esters of 1,4-benzenedicarboxylic acid (terephthalates) have applications as monomers in the manufacturing of several common synthetic fibers and plastics, and are used as additives. Since these additives are not chemically bonded to the polymer, they can migrate from the plastic into the environment under certain conditions [1,2]. Although phthalates and terephthalates have very low toxicity, the long exposure to these compounds may be associated with endocrine disruptions and may be linked to obesity [1]. Phthalates and terephthalates can be considered to be metabolic disruptors. For this reason, the analysis of phthalates and terephthalates has been of considerable interest and a very large body of publications is dedicated to this subject [3–18]. The analysis of phthalates has been frequently performed on food, toys, children's products, polymeric materials, and environmental samples such as air, water, and soil. Usual methods of analysis include gas chromatography with detection by flame ionization [3] or mass spectrometry [4,6,10–12,15,16], or liquid chromatography with detection by UV absorption [14]

---

* Corresponding author.
E-mail address: moldovs@rjrt.com (S.C. Moldoveanu).

https://doi.org/10.1016/j.chroma.2018.02.001
0021-9673/

Please cite this article in press as: S.C. Moldoveanu, R. Yerabolu, Critical evaluation of several techniques for the analysis of phthalates and terephthalates: Application to liquids used in electronic cigarettes, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.02.001

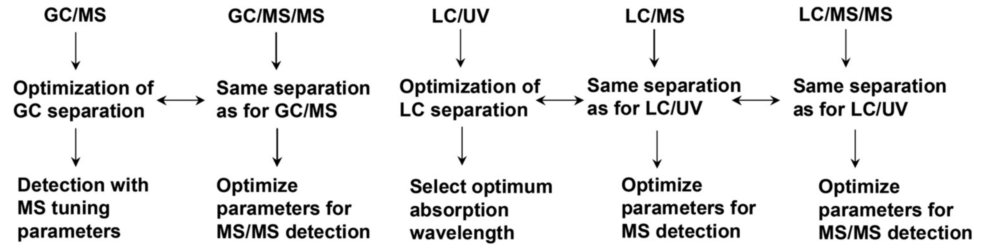

Fig. 1. Flow-diagram of the optimization process for each method.

or tandem mass spectrometry [5,7,9]. For enhancing the sensitivity of the analysis, a sample preparation step was frequently involved in the analysis. Among the sample preparation procedures that were utilized are ultrasound-assisted dispersive liquid–liquid microextraction [3,10], hollow fiber solvent bar microextraction [4], QuEChERS [5], pressurized liquid extraction [6], liquid–liquid extraction combined with solid phase extraction [9], head space solid phase microextraction [11], sorbent retention [12], stir-bead micro-solid phase extraction [13], dispersive solid-phase microextraction [14], etc. In this present study, the methods of analysis for eight phthalates and two terephthalates are applied to the liquids (e-liquids) used in electronic nicotine delivery systems (ENDS). The phthalates analyzed were diethyl, dibutyl, benzyl butyl, diphenyl, bis(2-ethylhexyl), di-*n*-octyl, diisononyl and diisodecyl. The terephthalates measured were dimethyl and bis(2-ethylhexyl). (The formulas for the analytes measured in this study and their exact mass M are indicated in Supplementary material 1).

The various methods evaluated in the present study did not use any sample preparation except the sample dissolution in an appropriate solvent. The study intended to determine the applicability of the separation and measurement technique for phthalates and terephthalates analysis, without involving cleanup or concentration in a sample preparation step.

2. Materials and method

2.1. Materials

Dimethyl terephthalate (CAS# 120-61-6), diethyl phthalate (CAS# 84-66-2), dibutyl phthalate (CAS# 84-74-2), benzyl butyl phthalate (CAS# 85-68-7), diphenyl phthalate (CAS# 84-62-8), bis(2-ethylhexyl) phthalate (CAS# 117-81-7), bis(2-ethylhexyl) terephthalate (CAS# 6422-86-2), di-*n*-octyl phthalate (117-84-0), diisononyl phthalates (mixture of isomers, CAS# 28553-12-0), and diisodecyl phthalate (bis(8-methylnonyl) benzene-1,2-dicarboxylate, CAS# 26761-40-0) as well as acetone, methanol and isopropanol were purchased from Sigma/Aldrich (St. Louis, Mo, USA). Dimethyl phthalate-3,4,5,6-d_4, and di-(2-ethylhexyl) phthalate-3,4,5,6-d_4 were purchased from C/D/N Isotopes Inc. (Point-Claire, Quebec, Canada). Water 18.2 mΩ/cm was obtained from a Barnsted Nanopure unit (Thermo Scientific Rockford, IL, USA).

2.2. Instrumentation

Several instruments were used in this study. The GC/MS analysis was performed on a 7890B/5977A GC/MS instrument (Agilent Technologies Inc., Wilmington, DE, USA). The GC/MS/MS analysis was performed on a 7890B/7010B instrument also from Agilent. The data from both instruments were processed using MassHunter Workstation software version B.08.00. The GC separation was performed on a DB-WAXEtr 30 m × 0.25 mm column with 0.25 μm film, from Agilent. The LC/UV instrument was a 1290 Infinity HPLC instrument (from Agilent), equipped with a binary pumping system, autoinjector, thermostatted column compartment, and DAD (diode array) detector. A similar instrument was used for LC/MS/MS except that the autoinjector was thermostatted and instead of the DAD detector, a Sciex 6500 triple quad instrument (AB Sciex, Framingham, MA, USA) was used. The LC/MS/MS system was controlled using Analyst 1.6.2 software, and the peak integration was performed with MultiQuant 3.0.1 software. The LC/MS work was performed on an Acquity H class UPLC instrument coupled with a PDA and a QDa detector (Waters Corporation, Milford, MA, USA). All LC separations were obtained on Acquity UPLC BEH Phenyl 2.1 × 100 mm columns with 1.7 μm particles (from Waters).

Table 1
GC operating parameters.

Parameter	Description	Parameter	Description
Initial oven temp.	35 °C	Inlet mode	Splitless
Initial time	3.0 min	Injection volume	2.0 μL
Oven ramp rate	20 °C/min	Carrier gas	Helium
Oven final first ramp	180 °C	Flow mode	Constant flow
Final time first ramp	0 min	Flow rate	1.5 mL/min
Oven ramp rate	5 °C/min	Nominal initial pressure	11.6 psi
Oven final temp.	260 °C	Purge flow to split vent at	1 min
Final time	8.75 min	Split vent	15 mL/min
Total run time	35 min	Outlet pressure	Vacuum
Inlet temp.	291 °C	Transfer line heater	280 °C

The high inlet temperature for the GC was selected above the boiling point of glycerin (290 °C) which is a main component of many e-liquids.

2.3. Overall methods description

The methods developed in this study do not use any sample preparation except for the dissolution of the sample in an appropriate solvent. The selectivity of each method was obtained solely based on the chromatographic separation that was optimized to avoid interferences, and on the detector selectivity. Sensitivity of each analytical method has been optimized (where feasible) by obtaining the best detector settings for generating the signal (highest signal to noise). A flow-diagram of the optimization process for each method is shown in Fig. 1. The further descriptions of individual methods provide details for the optimized parameters.

The goal of present study was to avoid any elaborate sample preparation. The minimal sample preparation in an analytical method is preferable, since it eliminates labor consuming steps and avoids additional sources of errors [19]. However, a sample preparation step for increasing the concentration of the analyte can be easily added for the methods further described in this study, if a method with lower sensitivity must be selected due to instrument availability restrictions.

2.4. GC separation

The optimized GC conditions for both the GC/MS and GC/MS/MS separations on the DB-WAXEtr 30 m × 0.25 mm column with 0.25 μm film are indicated in Table 1.

Please cite this article in press as: S.C. Moldoveanu, R. Yerabolu, Critical evaluation of several techniques for the analysis of phthalates and terephthalates: Application to liquids used in electronic cigarettes, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.02.001