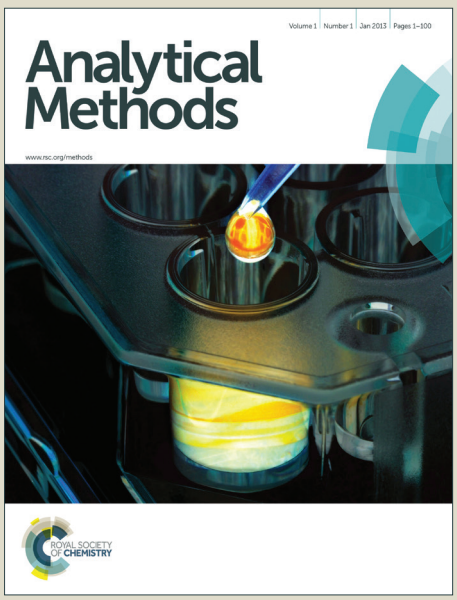
Analytical Methods

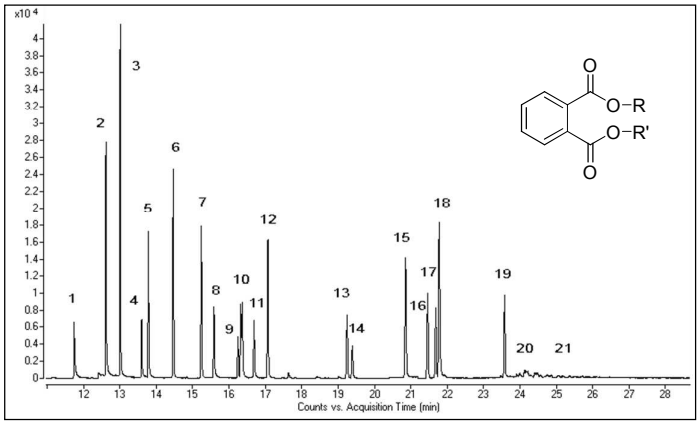
Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Development and validation of GC-MS/MS method to determinate 21 phthalate leachables in meter dose inhalers.



Analysis of 21 phthalate leachables in metered dose inhalers by gas chromatography tandem mass spectrometry

Xiaoxia Ye[a,b], Juan Ma[a], Yongjian Yang[b], Fang Jin[a]*

[a] Shanghai Engineering Research Center of Respiratory Medicine, Shanghai Institute of Pharmaceutical Industry，Shanghai 200437, China.

[b] Shanghai Institute for Food and Drug Control，Shanghai 201203, China

*Corresponding author:

Phone: +086-021-58368001 Fax: +86-21-58368001

E-mail address: jfsipi@163.com

ABSTRACT

For the first time, a gas chromatography tandem mass spectrometry method in MRM mode was developed and validated for separation and detection of 21 phthalate leachables in meter dose inhalers (MDI). The optimized method was reliable with high sensitivity and selectivity. Calibration curves were linear with correlation coefficients R^2>0.990, and the limit of detection (LOD) values for the analytes were in the range of 0.1-4.2 ng/ml except for Di(2-methoxyethyl) phthalate (DMEP), Di(2-ethoxyethyl) phthalate (DEEP), Di(2-n-butoxyethyl) phthalate (DBEP), for which LODs were around 10 ng/ml. Recovery ranged between 86.6 and 108.3%, and the RSD values of precision were within 7.72%. For the five MDI batches analyzed, 5 out of 21 phthalates were detected in each sample, including Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP) and Di(2-ehtylhexyl) phthalate (DEHP), with total phthalate amounts less than 260 ng /canister.

Keywords: Phthalate, GC-MS/MS, Leachable, Meter dose inhalers

1. Introduction

Phthalate esters (PAEs), known as 1,2-Benzenedicarboxylic acid esters, constitute a group of chemical compounds mainly used as plasticizers to improve the performance of plastics. Not forming covalent bonds with the plastics, PAEs can leach from the plastic into the drug formulations. An endocrine disrupting activity of PAEs, linked to estrogenic properties, has been described, [1-3] as well as their mutagenic and carcinogenic effects [4]. PAEs such as DEHP (Di(2-ehtylhexyl)phthalate) can cause respiratory reactions, and have been identified as important irritants and immunogens of respiratory syndromes including asthma/rhinitis, late respiratory systemic syndrome, pulmonary disease-anemia syndrome.[5,6] Based on DEHP animal exposure data and incomplete evidence from human epidemiologic studies, the Carcinogen Assessment Group of the US Environmental Protection Agency (EPA) classified plasticizers such as DEHP as "probable human carcinogens".[7] For the five phthalates BBP, DBP, DEHP, DEP, DNOP, the U.S. EPA Reference Dose (RfD) for exposure is 0.2，0.1，0.02，0.8 and 0.4 mg/kg/day， respectively.[8] Therefore, the total daily intake (TDI) of BBP, DBP, DEHP, DEP and DNOP should not exceed 12, 6, 1.2, 48 and 24 mg, respectively, for a 60 kg adult. Due to the potential risks for human health and environment, several PAEs have been included in the priority list of pollutants by different organizations. In Europe, the regulation (EC) NO. 1223/2009 [9] prescribes that some phthalates such as DBP or DEHP should phase out of cosmetics, while others such as diethyl phthalate (DEP) are still used without restrictions in many products. The FDA published a guidance for industry entitled "Limiting the Use of Certain Phthalates as Excipients in CDER-Regulated Products"[10] in October 2012 to restrict the

use of DBP and DEHP in pharmaceutical products. Following FDA's lead, the European Medicines Agency (EMA) restricted the use of phthalates in human medicines in 2013.

MDI (metered dose inhaler) is a type of drug/device combination products used in the treatment of a variety of lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), such as emphysema or chronic bronchitis and allergic rhinitis, as well as systemic diseases such as diabetes. [11] MDI consists of a solution or suspension formulation of a drug substance, hydrofluoroalkane (HFA) propellant that facilitates aerosol dose delivery, surfactant, co-solvents and other excipients that help to stabilize the formulation. The container closure and device system includes a metal canister to contain the pressurized formulation, a valve to meter the dose to patient, elastomeric components to seal the valve to the canister, and an actuator mouthpiece to facilitate patient self-dosing. HFA is a mid-polar solvent, with dissolvability as strong as that of dichloromethane. Due to HFA's high dissolvability, the organic chemicals, either purposefully added to the device materials (e.g. polymerization agents, antioxidants, plasticizers) or present in the materials as by-product of synthesis, may leach from device components into formulation, and thus be delivered to the patient.

According to the FDA “Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics”, inhalable aerosols are used as pharmaceutical products in which the likelihood of packaging component–dosage form interaction is the highest. Therefore, the amount of leachables such as phthalate plasticizers in MDI should be limited to safety range. Therefore, the development of reliable analytical methods

for PAEs detection is essential in MDI quality control.

Although many reports have been published on PAEs determination, most of them focus on plastic materials,[12,13] water,[14] vegetables,[15] cosmetics,[16] soil,[17] and wine.[18,19] For instrumental analysis, gas chromatography (GC) methods with flame ionization detection[12] or with mass spectrometry detection operating in single ion monitoring (SIM)[13-19] have been reported for PAEs determination. In addition, HPLC coupled to mass spectrometry and UV spectroscopy has been used in PAEs detection.[20,21] Most of these assays require various preconcentration techniques, such as liquid-liquid extraction,[13,17] stir bar sorptive extraction,[15] matrix solid-phase dispersion,[16] and solid-phase extraction.[12,14,18]

The multiple reaction monitoring mode (MRM) based on tandem mass spectrometry (MS/MS) combines high selectivity and sensitivity, and constitutes an excellent analysis tool for trace amounts in a complex matrix, where there are normally high intensities of background signals. The use of tandem quadrupole QqQ enables adequate precursor and product ions selection and allows reducing the chemical noise in chromatograms. In addition, the two steps of mass analysis in MS/MS systems based on QqQ offers the possibility of applying multiple reaction monitoring (MRM). Indeed, GC-MS/MS has been successfully used to determine low levels of leachable components in implantable medical devices.[22] To our knowledge, GC-MS/MS in MRM mode has not been reported for detection of PAEs trace amounts.

This paper focuses on the simultaneous quantitation of 21 PAEs by GC-MS/MS in MRM mode, covering most of common phthalate

plasticizers, in meter dose inhalers. To date, no study has been reported on detection of leachable PAEs in MDI by GC tandem mass spectrometry.

2. Experimental

2.1 Reagents

The 21 PAEs (chemical names and CAS numbers listed in Table.1) and DBP-d4 were all purchased from Dr. Ehrenstorfer GmbH (Germany); purity ranged from 99.5 to 100.0%. Analytical-reagent grade acetonitrile was provided by MERCK.

2.2 Instruments

The GC-MS system was composed of an Agilent 7890 gas chromatograph and an Agilent 7000A triple quadrupole (QqQ) mass spectrometer (MS/MS).

The GC inlet was operated in splitless mode, held at a temperature of 220°C and lined with a single taper deactivated inlet liner. Analytes were separated on a 30m DB-5MS (5%-dipenyl; 95%-dimethylpolysiloxane) capillary column with 0.25 mm internal diameter and 0.25 μm film thickness. The initial oven temperature was 60°C, held for 5 min, increased to 210°C at 10 °C/min, increased to 250°C at 5 °C /min, increased to 280°C at 10 °C/min and held 6 min (total run time 33.8 min). The helium carrier gas in column was maintained at a constant flow of 1 mL/min with an injection volume of 1 μL. The GC-MS/MS interface temperature was maintained at 280 °C.

Mass spectrometric ionization was undertaken in electron-impact ionization mode with an EI voltage of 70 eV and a source temperature of 230°C. The temperature of quadrupole analyzer was maintained at 150°C.

For collision induced dissociation (CID) experiments, ultra high purity nitrogen gas at 1.5 mL/min and helium quenching gas at 2.25 mL/min were used. The collision energy was optimized for individual chemicals to achieve a maximum peak response. The components were detected and quantified by multiple reaction monitoring (MRM) mode with the gain set to 30 for all analytes. In order to identify the most suitable transitions for MRM, analytical standards were initially analyzed in scan mode to determine suitable precursor ions in MS1 with a scan range of *m/z* 30 to *m/z* M+10 (where M is the mass of the compound of interest). Fragmentation of precursor ions in the collision cell was assessed by performing a production scan using the same mass range and scan time. Product ion intensity was optimized for each transition at different collision energies. All samples were run with a solvent delay of 3.5 min and the analytes were separated into 7 discrete time segments for MRM monitoring with dwell times ranging from 10 to 50 ms, depending on the time segment, to achieve 5 cycles/s across each peak for optimal quantification. All ions were monitored at wide resolution (1.2 amu at half height).

The ion transitions monitored for all analytes and collision energies for the method are presented in Table 1. The Agilent Mass Hunter v. B.04.01 software was used for data acquisition.

Table 1 GC-MS/MS method parameters for each analyte.

2.3 Method validation

DBP-d4 was used as the internal standard, dissolved and diluted to 1000 ng/mL with acetonitrile to prepare the IS stock solution. PAEs stock solutions (200 µg/mL) of each PAEs analytes were dissolved in

acetonitrile and serially diluted to prepare the working standards at 4, 20, 40, 80, 120, 160, and 200 ng/mL, except DMEP, DEEP and DBEP for which 40, 200, 400, 800, 1200, 1600, and 2000 ng/mL working standards were prepared.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by analysis of 1 μL of each standard dilution. Precision on standard solution were assessed at three concentration levels (40 ng/mL, 80 ng/mL, and 200 ng/mL). Accuracy was evaluated with the spiking recovery experiment. Spiked samples were prepared by adding PAEs stock solutions to matrix samples at three different concentration (40 ng/mL, 80 ng/mL, and 200 ng/mL).

2.4 Samples and analytical procedure

The metal canister of meter dose inhalers contains the pressurized formulation. Therefore, the pressure should be released before any assay. This was done by creating a small hole on the meter valve with an awl to discharge the propellant slowly, operating with caution to avoid jetting. When the propellant was completely discharged, MDIs were individually opened with a tubing cutter, the contents completely transferred into a 5 mL flask, followed by addition of 0.5 mL IS stock solution and acetonitrile to the mark. The solution was centrifuged for 5 min at 4000 rpm with a glass centrifuge tube, in case of unclear solution.

All glassware used was washed with acetone, rinsed with hexane and dried at 80°C for at least 2 hours, in order to eliminate phthalate contamination.

3. Results and discussion

3.1. GC separation

The separation conditions were optimized using a standard mixture of 21 PAEs at 1 μg/mL with scan mode. DB-1MS and DB-5MS capillary columns of 30 m×0.25 mm×0.25 μm were compared and better separation was achieved with DB-5MS. Therefore the latter was selected for further experiments. The temperature program was further optimized to achieve an efficient separation of the 21 PAEs. Fig.1A shows a chromatogram of a 120 ng/mL standard solution (DMEP, DEEP and DBEP at 1200 ng/mL). All PAEs except DINP and DIDP were chromatographically separated on DB-5MS. Both DINP and DIDP are complex mixtures of isomers, and the chromatographic signal consist of many peaks. Although a slight co-elution of DINP and DIDP was observed, DINP and DIDP were also effectively detected in our method, by monitoring different quantifier transitions, as showed in Fig.1B and 1C. As there are two chiral carbons in the BMPP molecule, BMPP appears as two incompletely separated isomer peaks in the chromatogram. Both isomers have almost the same mass fragmentations, and can't be differentiated by MRM mode, so they were co-detected and quantified.

After comparison between 220°C and 280°C, 220°C was selected as injection temperature, yielding a better injection precision.

Fig.1. GC-MS/MS chromatogram of 120 ng/ml standard solution.
A: Total ion chromatogram of mixed standard solution
1: DMP, 2: DEP, 3: DIPrP, 4: DAP, 5: DPrP, 6: DIBP, 7: DBP& DBP-d4, 8: DMEP, 9: DIPP 10: BMPP, 11: DEEP, 12: DPP, 13: BBP, 14: DHP, 15: DBEP, 16: DCHP, 17: DEHP, 18: DPHP, 19: DNOP, 20: DNP, 21: DIDP
B: MRM chromatogram of DNP (293 → 149)
C: MRM chromatogram of DIDP (307 → 149)

3.2 Optimization of MS parameters

In order to enhance the sensitivity of detection, mass spectrometry parameters were optimized. Careful attention was paid to the selection of

suitable MRM transitions to produce product ions of high intensity and minimal background noise. Analytical standards were initially analyzed in scan mode, fragmentation with higher *m/z* and response was picked out as precursor ion. Then the fragmentation of precursor ions in the collision cell was assessed by performing a production scan, fragmentation with higher *m/z* and response was picked out as product ion. Compared by the response and background noise, quantitation transition and qualitation transition with different precursor may be selected. Take DIPP for example, the precursor ion of the quantitation and qulitation transition were 237 and 149 respectively. Response result in different collision energy may vary to a different degree. Therefore, product ion formation was optimized at variable collision energies (between 5 and 25ev) once the transitions were selected, to achieve high sensitivity. The response of transition 149→121 of DIPP at different collision energy levels is shown in Fig.2. The highest response was achieved at 15ev. Dwell times were adjusted to provide 5 cycle/s for sufficient peak scan. As the response of the qualifier transition 293→71 of DINP is low, another qualifier transition 293→167 was selected at the same time, in order to improve specificity. The same is valid for DIDP.

Fig.2 Effect of collision energy (ev) on the transition 149→121 of DIPP.

3.3 Method validation

The optimized method was validated for linearity, detection limits, quantification limits, precision and accuracy. Calibration curves were constructed for each analyte, plotting (peak area of analyte quantifier transition)/(peak area of IS transition) versus concentration. The calibration curves were linear for all the standards with regression values (R^2) ranging from 0.9902 to 0.9985 (Table.2).

Although all possible precautions were taken, the presence of trace phthalates in solvent could not be avoided. The limit of detection (LOD) and quantification (LOQ) were calculated based on a signal-to-noise ratio of 3 and 10 (Table 2), respectively, excluding DEP, DIBP, DBP, and DEHP, detected in the blank solvent (acetonitrile). LODs and LOQs for these compounds were estimated as the average amount of analyte giving a response that is the signal plus 3 or 10 times the standard deviation, respectively. LODs of the PAEs studied were around 1.0 ng/mL, except for DMEP, DEEP ,DBEP, DINP and DIDP. The respective values are listed in Table 2. Due to the high selectivity of MRM mode, the noise in the MRM chromatogram (307→149) of DINP tends to be zero. Therefore, detection was still possible even at level as low as to 3.9 ng/mL, despite the relatively low height of DINP peak (Fig.1). The same is valid for DIDP. LOQs ranged from 0.4 to 8.3 ng/mL, with the exception of DMEP, DEEP and DBEP (36.5~41.2 ng/mL).

Precision on standard solution was studied within day (n=6) and between days (n=6) at low, medium, and high levels (40, 80, 160 ng/mL). Intraday precision expressed as the relative standard devisions (RSDs) for all analytes were listed in Table 2, varied from 1.52 to 7.72%. Interday RSDs values range from 1.64 to 8.45%.

Table 2 Characteristics of the optimized method.

The matrix effects of the 21 PAEs were estimated by comparing the corresponding response factors for the spiked sample to those of pure standards at two levels (40 ng/mL and 160 ng/mL). Matrix effects between 89.9 to 110.3% were observed, suggesting the minor matrix effects at acceptable levels. Therefore, the standard in acetonitrile was used to calculate the linearity and sample assay.

Method accuracy were evaluated with spiking experiments at three levels (40, 80, 160 ng/mL, n=3) (Table.2). As shown in Table 2, recovery calculated by calibration curves ranged from 86.6 to 108.3%.

3.4 Analysis of samples

The developed method was applied to five meter dose inhaler batches. Three samples were prepared and analyzed respectively for each batch. The analytes were identified by comparing the retention time and the response ratio of qualifier ion to target ion in samples and standard solutions. Five PAEs were found in all meter dose inhalers, including DMP, DEP, DIBP, DBP and DEHP, and the others were not detected in the analyzed samples. The results are summarized in Table 3. The total PAEs amounts ranged from 174.4 to 252.5 ng/canister for the five MDI. There are 200 doses in one canister, and a patient may administer up to 4 doses per day. Accordingly, the patient would inhale 3-5 ng of PAEs with the drug daily, far less than the allowed intake for the most dangerous PAEs: DEHP, 0.12 mg/day. DEHP amounts range from 41. 8 to 135.2 ng/canister, accounting for 24~54% of the total PAEs. These data suggest that DEHP is the main phthalate plasticizer in meter valve materials. Due to the special inhalation route, in which the chemical may directly enter into the blood system, PAEs leachable in MDI should be controlled within the safe range. Although the amounts of PAEs in MDI are far less than reference doses proposed by the U.S. EPA, MDI developers and manufacturers should still pay close attention to leachable PAEs. Also, this research field should be intensified to ensure the safety of MDI.

Table 3 Contents (ng/canister) of MDI products (n=3).

4. Conclusion

This paper describes the development and validation of a reliable GC-MS/MS method for the determination of PAEs leachable. 21 PAEs could be simultaneously analyzed in this optimized method. The method was applied to five batches of MDI, and five phthalate plasticizers (DMP, DEP, DIBP, DBP and DEHP) were found in MDI products as leachable. MDI developers and manufacturers should pay close attention to the PAEs leachable in MDI products to ensure quality and safety of their products.

References

[1] R. Benson, Hazard to the developing male reproductive system from cumulative exposure to phthalate esters-dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, diethylhexyl phthalate, dipentyl phthalate, and diisononyl phthalate. *Regul. Toxicol. Pharm*, 2009,53, 90-101.

[2] T. Stroheker, N. Cabaton, G. Nourdin, J.F Regnier, J.C. Lhuguenot, M.C. Chagnon, Evaluation of anti-androgenic activity of di-(2-ethylhexyl)phthalate Toxicology, 2005,208,115-121.

[3] M. Matsumoto, M. Hirata-Koizumi, M. Ema, Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction. Regul Toxicol Pharm, 2008,50 , 37-49.

[4] H. JW, Carcinogenicity of general-purpose phthalates:structure-activity relationships Drug Metab Rev. 1989,21,55.

[5] S.H. Sandra , R Brenda shaw, A. Wu , The clinical effects of plasticizers, antioxidants, and other contaminants in medical polyvinylchloride tubing during respiratory and non-respiratory exposure.Clin.Chim.Acta, 2001,304, 1-8.

[6] A.R. Bardana, Occupational asthma secondary to low molecular weight agents used in the plastic and resin industries. Eur. J. Resp. Dis, 1983,64 , 241-251.

[7] C.O Schulz, Assessing human health risks from exposure to di(2-ethylhexyl) phthalate (DEHP) and related phthalates. Drug. Metab. Rev, 1989,21 , 111-120.

[8] http://www.epa.gov/teach/chem_summ/phthalates_summary.pdf. Phthalate.

[9] Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products (recast), Off. J. Eur. Union L342 (2009)59.

[10] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances//ucm 330792.htm Guidance for Industry Limiting the Use of Certain Phthalates as Excipients in CDER-Regulated Products(2012).

[11] S. Newman, P. Anderson, P.Byron, R. Dalby, J.Peart, Respiratory Drug Delivery Essential

Theory&Practice.2009. ISBN 978-7-122-10756-5

[12] X. Li, Z. Zeng, Y. Chen, Y. Xu, Determination of phthalate acid esters plasticizers in plastic by ultrasonic solvent extraction combined with solid-phase microextraction using calix[4]arene fiber. Talanta.,2004, 63, 1013-1019.

[13] T. Niino, T. Ishibashi, T. Itho, S. Sakai, H. Ishiwata, T. Yamada, S. Onodera, Simultaneous determination of phthalate di- and monoesters in poly(vinylchloride) products and human saliva by gas chromatography mass spectrometry.J. Chromatogr. B. 2002,780, 35-44.

[14] Y.B Luo, B.F. Yuan, Y.Q. Feng, Fast microextraction of phthalate acid esters from beverage, environmental water and perfume samples by magnetic multi-walled carbon nanotubes. Talanta. 2012, 123-131.

[15] J. I.Cacho, N.Campillo,P.Viňas，M.Hernandez-córdoba, Determination of alkylphenols and phthalate esters in vegetables and migration studies from their packages by means of stir bar sorptive extraction coupled to gas chromatography mass spectrometry.J. Chromatogr. A. 2012, 1241,21-27.

[16] M. Llompart, M. Celeiro, J. Pablo Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, Analysis of plasticizers and synthetic musks in cosmetic and personal care products by matrix solid-phase dispersion gas chromatography mass spectrometry. J. Chromatogr. A. 2013,1293, 10-19.

[17] S. Kong, Y. Ji, L. Liu, L. Chen, X. Zhao, J. Wang, Z. Bai, Z. Sun, Diversities of phthalate esters in suburban agricultural soils and wasteland soil appeared with urbanization in China. Environ. Pollut. 2012, 170, 161-168.

[18] M. Del Carlo, A. Pepe, G. Sacchetti, D. Compagnone, D. astrocola, A. Cichelli, Determination of phthalate esters in wine using solid-phase extraction and gas chromatography mass spectrometry. Food. Chem. 2008,111, 771-777.

[19] G. Cinelli, P. Avino, I. Notardonato, A. Centola, M.V. Russo, Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersive liquid- liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography mass on trap mass spectrometry. Analy. Chim. Acta. 2013, 769, 72-78.

[20] A. Mtibe, T.A.M. Msagati, A.K. Mishra, B.B. Mamba, Determination of phthalate ester plasticizers in the aquatic environment using hollow fibre supported liquid membranes. Phys. Chem.Earth. Parts A/B/C 2012,50-52,239-242.

[21] L. Lu, Y. Hashi, Z.H. Wang, Y. Ma, J.M. Lin, Determination of phthalate esters in physiological saline solution by monolithic silica spin column extraction method. J.Pharma. Analy. 2011,1, 92-99.

[22] Barbara L.A, Askim F.S, Venkat Narayan, Xiande Wang, Lori. A, Gyorgy Vas. Stir bar sorptive extraction combined with GC-MS/MS for determination of low level leachable components from implantable medical devices. J.Pharm. Biomed, Anal, 2013,74,162-170.

396 **Table 1：** GC-MS/MS method parameters for each analyte

Key	Compound name	CAS	Retention Time(min)	Quantitation Transition(ev)*	Qualitation Transition(ev)*
DMP	Dimethyl phthalate	131-11-3	11.7	163→133 (10)	163→135 (25)
DEP	Diethyl phthalate	84-66-2	12.6	149→121 (15)	177→149 (25)
DIPrP	Diisopropyl phthalate	605-45-8	13.0	149→121 (15)	209→149 (5)
DAP	Diallyl phthalate	131-17-9	13.6	132→104 (15)	189→41 (25)
DPrP	Dipropyl phthalate	131-16-8	13.8	149→121 (15)	209→149 (5)
DIBP	Diisobutyl phthalate	84-69-5	14.5	149→121 (15)	223→149 (5)
DBP	Dibutyl phthalate	84-74-2	15.2	149→121 (15)	223→149 (5)
DBP-d4	Dibutyl phthalate-d4	93952-11-5	15.2	227→153 (15)	209→153 (15)
DMEP	Di(2-methoxyethyl) phthalate	117-82-8	15.6	149→121 (15)	207→59 (5)
DIPP	Diisopentyl phthalate	605-50-5	16.3	149→121 (15)	237→149 (5)
BMPP	Bis(4-methyl-2-pentyl) phthalate	146-50-9	16.4	167→149 (15)	251→167 (10)
DEEP	Di(2-ethoxyethyl) phthalate	605-54-9	16.7	193→149 (15)	149→121 (15)
DPP	Dipentyl phthalate	131-18-0	17.1	149→121 (15)	237→149 (5)
DHP	Dihexyl phthalate	84-75-3	19.3	149→121 (15)	251→149 (20)
BBP	Benzyl butyl phthalate	85-68-7	19.4	149→121 (15)	206→149 (5)
DBEP	Di(2-n-butoxyethyl) phthalate	117-83-9	20.9	149→121 (15)	193→121 (5)
DCHP	Dicyclohexyl phthalate	84-61-7	21.5	149→121 (10)	249→149 (25)
DEHP	Di(2-ehtylhexyl)phthalate	117-81-7	21.7	149→121 (10)	279→149 (20)
DPHP	Diphenyl phthalate	84-62-8	21.8	225→77 (25)	225→197 (10)
DNOP	Dinoctyl phthalate	117-84-0	23.6	149→121 (15)	279→149 (20)
DINP	Diisononyl phthalate	28553-12-0	24.1	293→149 (15)	293→71 (10) 293→167 (5)
DIDP	Diisodecyl-o-phthalate	26761-40-0	26.1	307→149(15)	307→71 (10) 307→167 (5)

397 *Listed in the brackets were the collision energy.

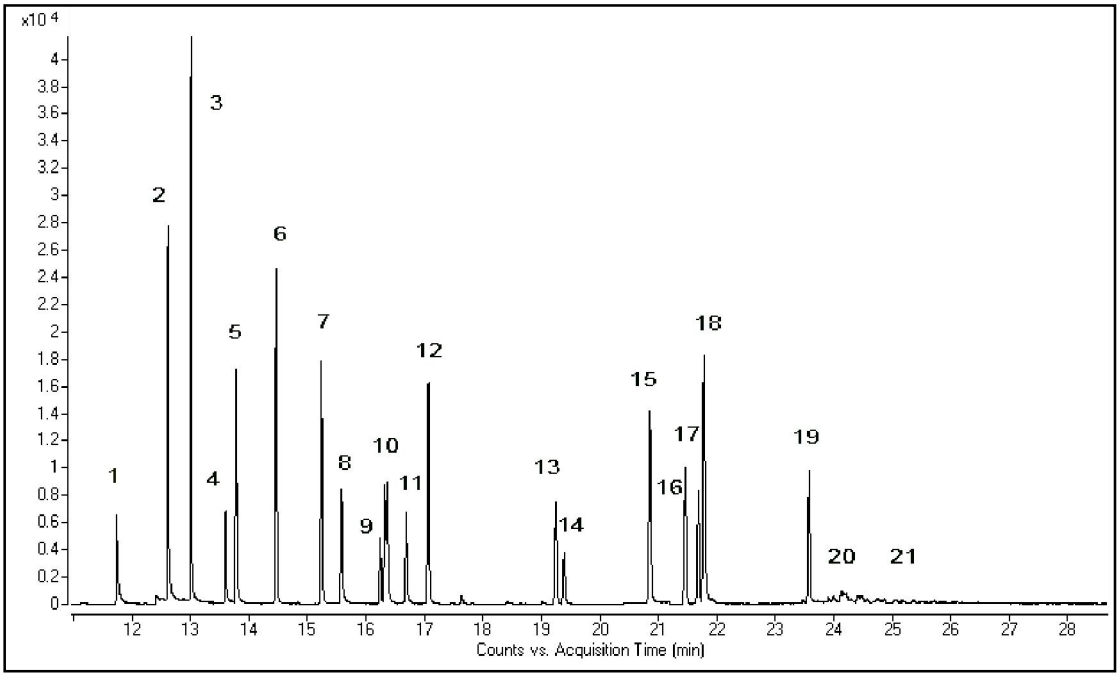
398 **Table 2:** Characteristics of the optimized method.

compound	R^2	LOD	Intraday Precision, RSD(%)(n=6)			Recovery Average(%) ± SD(n=3)		
		ng/mL	40 ng/mL[a]	80 ng/mL[b]	160 ng/mL[c]	40 ng/mL[a]	80 ng/mL[b]	160 ng/mL[c]
DMP	0.9913	0.2	4.08	3.86	4.24	92.1 ± 2.13	102.3 ± 0.45	90.7 ± 3.78
DEP	0.9959	1.0	4.34	2.20	4.66	98.3 ± 2.22	98.9 ± 0.64	93.4 ± 3.33
DIPrP	0.9973	0.2	3.28	1.52	4.08	101.4 ± 4.14	100.5 ± 1.03	99.4 ± 1.52
DAP	0.9951	1.1	1.83	1.75	4.37	102.9 ± 2.73	98.3 ± 1.23	100.2 ± 2.56
DPrP	0.9919	0.4	2.05	1.40	4.22	99.9 ± 4.44	101.3 ± 1.44	97.7 ± 1.32
DIBP	0.9929	0.2	1.53	2.01	3.85	105.4 ± 2.04	98.7 ± 1.43	101.8 ± 1.67
DBP	0.9985	0.2	2.15	2.65	3.88	107.5 ± 3.57	100.2 ± 2.52	107.6 ± 1.68
DMEP	0.9902	10.3	4.18	6.25	3.10	103.0 ± 4.06	96.8 ± 1.78	108.3 ± 1.64
DIPP	0.9983	1.0	3.47	3.49	3.49	92.7 ± 1.54	96.8 ± 2.90	90.9 ± 1.79
BMPP	0.9985	0.2	2.08	2.09	3.60	89.2 ± 2.89	95.5 ± 2.01	94.6 ± 1.56
DEEP	0.9968	10.1	3.45	6.28	4.32	93.1 ± 2.67	98.6 ± 2.47	101.4 ± 1.02
DPP	0.9973	0.4	2.66	4.23	4.55	88.8 ± 2.73	101.7 ± 1.64	100.6 ± 1.89
DHP	0.9967	0.4	3.35	4.50	4.27	87.5 ± 2.44	93.3 ± 2.33	88.1 ± 1.02
BBP	0.9979	1.0	3.17	4.25	4.78	88.5 ± 3.43	92.7 ± 3.32	86.6 ± 2.43
DBEP	0.9956	9.1	4.85	5.90	4.90	89.9 ± 3.87	99.6 ± 1.52	101.8 ± 1.89
DCHP	0.9969	1.0	3.16	4.42	4.38	89.0 ± 3.01	87.1 ± 5.42	92.2 ± 2.43
DEHP	0.9959	1.1	3.00	5.81	3.37	102.2 ± 2.89	102.8 ± 6.44	91.7 ± 3.22
DPHP	0.9955	0.1	4.78	7.11	4.76	92.1 ± 4.53	95.5 ± 1.68	97.1 ± 4.51
DNOP	0.9977	1.0	4.86	7.72	4.39	97.7 ± 4.21	96.3 ± 0.40	89.8 ± 5.32
DINP	0.9956	3.9	7.44	5.81	5.75	99.5 ± 6.23	106.2 ± 1.53	94.1 ± 5.21
DIDP	0.9920	4.2	6.82	7.14	7.01	104.4 ± 4.02	94.7 ± 1.71	102.6 ± 5.73

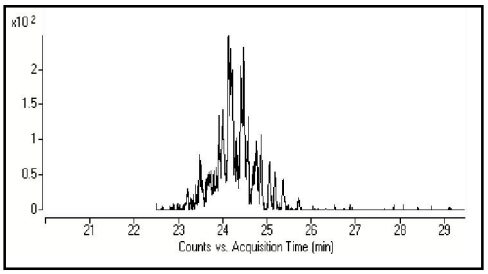
Table 3： Contents (ng/canister) of MDI products (n=3).

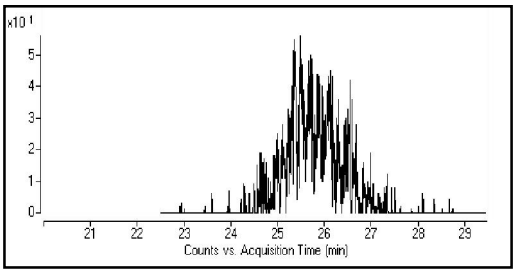
compound	MDI 1 mean ± SD	MDI 2 Mean ± SD	MDI 3 Mean ± SD	MDI 4 Mean ± SD	MDI 5 Mean ± SD
DMP	23.5 ± 1.21	29.0 ± 4.01	60.2 ± 6.28	17.3 ± 1.03	17.4 ± 0.54
DEP	21.9 ± 1.82	20.2 ± 0.74	22.1 ± 2.31	26.5 ± 8.32	22.6 ± 3.63
DIPrP	ND	ND	ND	ND	ND
DAP	ND	ND	ND	ND	ND
DPrP	ND	ND	ND	ND	ND
DIBP	34.9 ± 7.90	59.6 ± 6.68	25.3 ± 7.87	27.8 ± 3.82	64.4 ± 7.23
DBP	37.0 ± 6.93	33.3 ± 6.76	43.1 ± 6.82	42.4 ± 0.54	28.4 ± 5.13
DMEP	ND	ND	ND	ND	ND
DIPP	ND	ND	ND	ND	ND
BMPP	ND	ND	ND	ND	ND
DEEP	ND	ND	ND	ND	ND
DPP	ND	ND	ND	ND	ND
DHP	ND	ND	ND	ND	ND
BBP	ND	ND	ND	ND	ND
DBEP	ND	ND	ND	ND	ND
DCHP	ND	ND	ND	ND	ND
DEHP	135.2 ± 7.56	82.5 ± 4.04	87.5 ± 2.67	62.5 ± 7.88	41.8 ± 6.77
DPHP	ND	ND	ND	ND	ND
DNOP	ND	ND	ND	ND	ND
DINP	ND	ND	ND	ND	ND
DIDP	ND	ND	ND	ND	ND
SUM	252.5 ± 10.53	224.4 ± 13.38	238.2 ± 19.48	176.3 ± 4.11	174.4 ± 14.13

ND: not detected



A





B C

Fig.1. GC-MS/MS chromatogram of 120 ng/ml standard solution.
A: Total ion chromatogram of mixed standard solution
1: DMP, 2: DEP, 3: DIPrP, 4: DAP, 5: DPrP, 6: DIBP, 7: DBP& DBP-d4, 8: DMEP, 9: DIPP 10: BMPP, 11: DEEP, 12: DPP, 13: BBP, 14: DHP, 15: DBEP, 16: DCHP, 17: DEHP, 18: DPHP, 19: DNOP, 20: DNP, 21: DIDP
B: MRM chromatogram of DNP (293 → 149)
C: MRM chromatogram of DIDP (307 → 149)

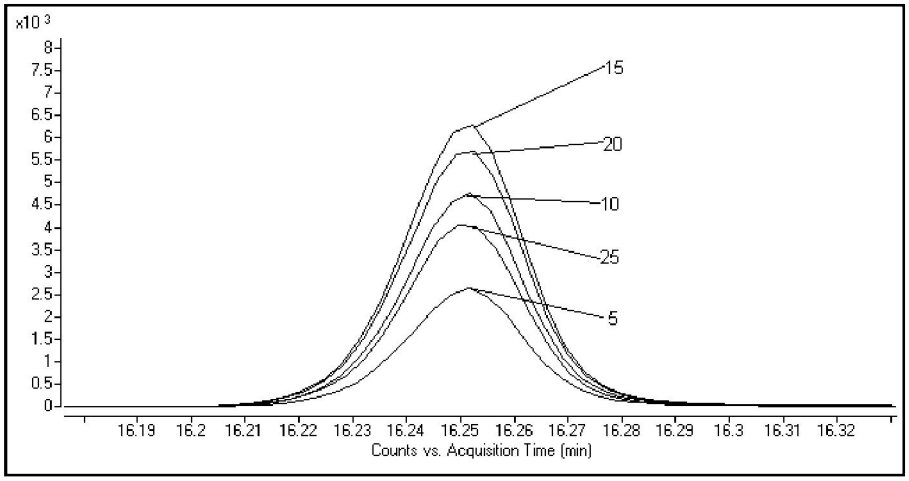


Fig.2. Effect of collision energy on the transition 149 → 121 of DIPP.