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Evaluation of Phthalate Levels in Toys in Egyptian Market

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THE occurrence and levels of three common phthalates in toys and children products sold in the Egyptian market were investigated for first time. The investigated phthalates were diethyl phthalate (DEP), dibutyl phthalate (DBP) and dioctyl phthalate (DOP). Phthalates were extracted from samples by solid-liquid extraction followed by cleanup process. The samples were analyzed using HPLC system coupled with UV detector. The results showing presence of phthalates in most samples with levels exceeds the allowed values. Diethyl phthalates varied from 0.002% to 0.62% (in 65% of samples), dibutyl phthalate varied from 0.008% to 0.93% (in 60% of samples) and dioctyl phthalate varied from 0.02% to 1.15% (in 90% of samples). Raman spectra was used as reference method to verify the presence of the tested plasticizers (DEP, DOP and DBP) into the toy samples.

Keywords: Phthalates, Determination, HPLC Analysis, Children toys.

Introduction

For decades, phthalate compounds were considered the most popular plasticizers used in many industries like inks, adhesives, paints, cosmetics, plastics and as asphalt modified [1]. They are included to packaging materials to increase their flexibility, transparency and durability. This means that phthalates are commonly present in many surrounding objects with direct contact with human. Since phthalates are not chemically bound to plastics, they can easily migrate to surrounding air, soil, dust and food causing micro pollution [2, 3].

Plasticizers are added during the production of polymers to change the physical properties making them more flexible and elastic. The action of plasticizer is to reduce the glass transition temperature (T_g) to below room temperature [4]. The plasticized polymers can be used widespread in many applications more than rigid polymers [5]. The difference in structure between rigid polymer and plasticized polymer is shown in Figure 1. The plasticizer needs to be well mixed with polymer matrix until they are dissolved together for more effective results [6].

Majority of plasticizers are organic esters and the properties required for final product will determine the plasticizer to be added [4]. They can be classified into primary and secondary types. Primary plasticizer is chemicals that interact with polymer and considered the basic formulation of plasticizers. Secondary plasticizers have less compatibility with polymers and act with primary plasticizers as additives to improve certain properties in polymers. Phthalates are the ortho-isomer of 1, 2-benzenedicarboxylic acid with two side chains of alkyl, benzyl, phenyl, cycloalkyl, or alkoxy groups. They can divide into low molecular and high molecular based on carbon number of their alcohol. They have the general formula as illustrated below:

Phthalates are considered one of the most common human endocrine disturbing chemicals spread worldwide [7]. Accordingly, many authorities have been established to control and spread of certain phthalates [8]. Phthalate's effect on health has great concern of researchers, firstly on animals intensive studies showing that dibutyl phthalate(DBP) and Bis(2-ethylhexyl) phthalate(DEHP) have chronic effect on

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development and reproduction [9, 10]. In human studies, Colon et al. suggested a relation between phthalate levels and abnormal reproductive phenomena in Pureto Rican girls [11]. Also, Kobrosly et al. found that high levels of phthalates metabolites in urine associated with behavioral problems in children [12]. Phthalate exposure not only affects the persons themselves but also affect their babies [12]. In case of pregnant mothers Cantonwine et al. found a relation between high levels of phthalates metabolites in early pregnancy and decreased cervical length which increase the risk of preterm birth [13]. Also, Herberth et al. suggested a relation between high levels of maternal phthalates metabolites in urine during pregnancy and lowering numbers of T-cells cause low immunity in children [14]. Studies have also shown that exposure to phthalates is associated with sexual hormones disorder [15] and lower semen quality [10, 16].

One of the main sources of children exposure to phthalates is the plastic toys due to mouth activities including sucking and chewing. By increasing period of playing with toys the more phthalate metabolites were found in urine [17]. So, it's important to maintain the levels of phthalates in toys under control.

In Egypt, the level of phthalates in toys was not previously investigated. The aim purpose of this study was to examine the occurrence and quantify the levels of phthalates in toys locally traded in the Egyptian market. The Egyptian standard specifications for toys put limits for certain phthalates of acute health effect [18, 19]. The data mentioned in these specifications was used as a reference in comparing the results whether they comply or not with limits and the possible consequent health effect.

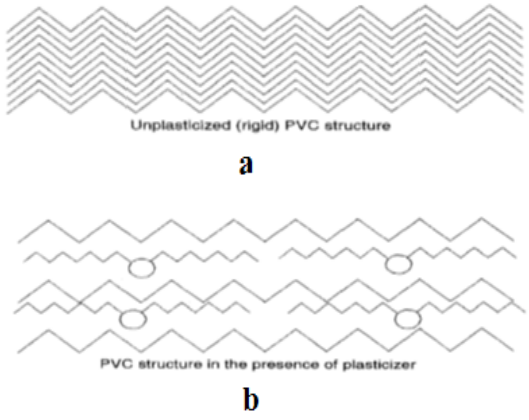


Fig. 1. Structure of unplasticized (a) and plasticized (b) PVC.

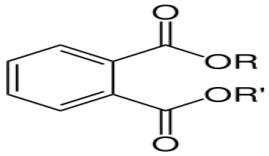


Fig.2. General structure formula of phthalates.

Materials and Methods

Samples

20 new children (0-6 years) toy samples for such as: Whistle, plastic trucks, rattle toy, plastic horse carriage, water bottles and girl dolls were purchased from local market in Cairo in May2017. Pacifiers and baby suckers were purchased from pharmacies in 10th of Ramadan City in Jan 2018. The sample selection basis considers the screening for common child products consumed in Egypt and may contain phthalates.

Reagents and material

Diethyl phthalate (DEP) and dioctyl phthalate (DOP) standard solutions were purchased from (Sigma Aldrich, USA). Dibutyl Phthalate (DBP) standard solution and acetonitrile HPLC grade were purchased from (Loba-Chemie, India). Dichloromethane (DCM), n-hexane of analytical grade and activated silica gel (60-120) mesh were purchased from (Advent-ChemBioPvt.LTD, India). Distilled water was freshly prepared in Lab. Sodium Sulfate anhydrous was purchased from (Adwec Chemicals, Egypt). Stock standard solution was prepared by dissolving 50 mg of each phthalate standard in 50 ml of acetonitrile, and then it was stored in refrigerator until use. Further dilutions by acetonitrile were done to obtain working standard solution mixture of 1, 3, 6 and 10µg/ml for calibration curves.

Sample preparation and extraction:

In order to minimize interference only glassware tools were used in all steps of procedures and plastic devices were avoided. Glassware was rinsed several times before use by hexane followed by ethyl acetate then dried in an oven at 200 ˚C for 1 hour. Portions of each PVC sample were cut into small pieces less than 2 mm. About 2.5 g of the obtained pieces were weighed accurately then dissolved in 25 ml of DCM by shaking for several minutes. PVC-free toys which was not dissolved in DCM was put in water path at 50 ˚C for 3 hours then they were left in DCM for 24 h at room temperature before cleaning up.

Clean up and separation:

The cleanup and separation process were performed as described before [20] with some modifications. Glass chromatographic column (4 cm x 50 cm) equipped with glass stopcock and G4 separator. 10 g of activated silica gel (60-120 mesh) was put in the column and kept until setting. Then, 1 cm layer of anhydrous sod sulfate was added to the top of silica gel. 20 ml n-hexane was used for re-elute the column at a rate of 2 ml/minute. 2ml of the sample was accurately transferred to the column and then additional 2 ml of hexane were added to complete the transfer of the sample down the column. 20 ml of n-hexane was used to elute the column for non-polar substituent at a rate of 1 ml/ minute. 40 ml ethyl acetate was used to elute the phthalates. The elute was concentrated to 1 ml by using water bath at 40˚ C. Then, it was left to complete dryness at room temperature. The residue was re-dissolve in 1 ml acetonitrile for HPLC analysis.

Instruments

The instrumental analysis of phthalates was carried out using ISCO model 2350 HPLC system coupled with UV detector (Central Lab., Zagazig University, Zagazig, Egypt). Transmitting was performed at 226 nm. Hypersil BDS (250 cm x 4.6 mm) 5U C_{18} capillary column was used for chromatographic separation. The flow rate was 1.0 ml/min, 10 µl was used as injection volume sample to HPLC system. Isocratic elution condition was used; the mobile phase was acetonitrile and water (90:10) [21]. The Raman spectra was obtained using Raman Spectrometer Horibia Lab RAM HR Evolution (Center of nanotechnology, Cairo university, Giza, Egypt). The measurements were performed according to previous procedure [22].

Results and Discussion

Analytical Results

The analysis was tested firstly at wavelength 254 nm according to previously method mentioned by Adewuyi and Olowu [20]. It was observed that peaks of DBP and DOP were overlapped. When wavelength was changed to 226 nm, the resulted peaks were satisfactory due to the absence of overlapping. Also, the response was higher as the peak area resulted was higher as shown in figure 3 and figure 4. Accordingly, all measurements were preferred to be performed at 226 nm.

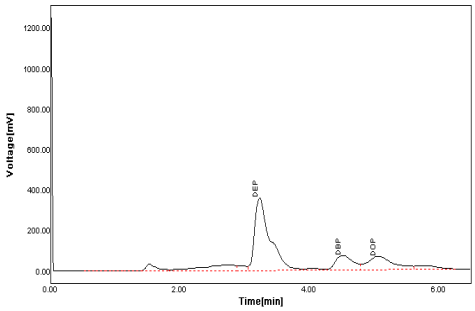


Fig. 3. Chromatogram of standard solution 10 ppm of DEP, DBP and DOP at wavelength 226 nm.

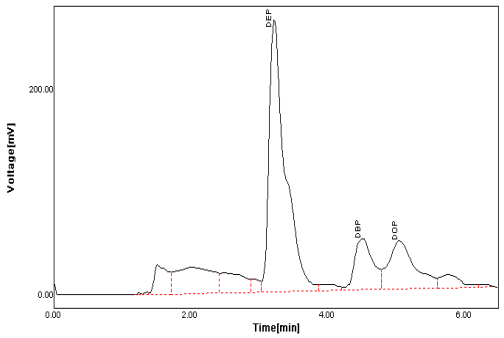


Fig. 4. Chromatogram of standard solution 10 ppm of DEP, DBP and DOP at wavelength 254 nm.

TABLE 1. Analytical results of DEP, DBP and DOP standard solution 10 ppm measured at 226 nm.

No.	Name	t_R, (min)	Area, (mV*s)	Height, (mV)
1	DEP	3.2333	5841.8032	358.1754
2	DBP	4.5167	1306.4990	69.3064
3	DOP	5.0500	1821.6139	65.4285
Sum			8969.9160	492.9104

TABLE 2. Analytical results of DEP, DBP and DOP standard solution 10 ppm measured at 254 nm.

No.	Name	t_R, (min)	Area[mV*s]	Height[mV]
1	DEP	3.2167	4329.7598	264.2312
2	DBP	4.5167	928.4319	49.8527
3	DOP	5.0500	1242.1887	46.5373
Sum			6500.3799	360.6213

The mass percentage of phthalates in toys (w/w) was calculated according to equation [22]:
Percentage [Phthalate] = % Phthalate (w/w) = [(C x V x D) / (W x 1000)] x 100
Where
C = Concentration of phthalate (in µg/ml)
V = Total volume of DCM of phthalate extraction method
D = Dilution factor
W = Weight of sample collected (in mg)
The limits of phthalates in toys are 0.1% w/w (FDA guidance, 2012).

TABLE 3. Concentration of phthalates in toy samples.

Sample	DEP, (µg/g)	DBP, (µg/g)	DOP, (µg/g)
A1	35.15	ND	1.38
A2	20.61	180.02	20.94
B1	15.70	6.85	ND
B2	ND	85.01	49.01
B3	ND	15.80	ND
B4	ND	743.99	37.93
C1	ND	10.44	7.68
C2	ND	ND	ND
C3	60.25	11.84	ND
D1	6.64	ND	100.1
D3	49.14	ND	23.10
D4	ND	ND	ND
E1	37.13	157.02	31.64
E2	ND	ND	49.58
E3	189.70	ND	273.76
F1	492.42	72.28	24.86
F2	21.04	53.96	9.20
F3	334.32	175.00	26.94
F4	16.49	225.61	38.84
G1	117.98	ND	134.51

TABLE 4. Percentage of phthalates in toy samples.

Sample	DEP, % w/w	DBP, % w/w	DOP, % w/w	Total phthalates, %
A1	0.0879	ND	0.0035	0.0914
A2	0.0258	0.225	0.0262	0.277
B1	0.0196	0.0086	ND	0.0282
B2	ND	0.1063	0.0613	0.1676
B3	ND	0.0198	ND	0.0198
B4	ND	0.93	0.0474	0.9774
C1	ND	0.0131	0.0096	0.0227
C2	ND	ND	ND	ND
C3	0.0753	0.0148	ND	0.0901
D1	0.0028	ND	0.0417	0.0445
D3	0.0614	ND	0.0289	0.0903
D4	ND	ND	ND	ND
E1	0.0928	0.3926	0.0791	0.5645
E2	ND	ND	0.124	0.124
E3	0.4743	ND	0.6844	1.1587
F1	0.6155	0.0904	0.0311	0.737
F2	0.0263	0.0675	0.0113	0.1051
F3	0.4179	0.2188	0.0337	0.6704
F4	0.0412	0.564	0.0971	0.7023
G1	0.295	ND	0.3363	0.6313

To ensure results the samples were analyzed using Raman spectrometer. FT-Raman spectra for pure phthalates were firstly investigated. The resulted spectra are shown in figure 5, which have six characteristic bands for phthalates in the range between (500-1800) cm^{-1}. The bands at 3074,1600, 1580, 1040, and 652 cm are due to vibration of ortho-phenyl group. On the other hand, the peak at 1726 cm was due to the carbonyl (C=O) stretching vibration of the ester group [23]. The samples were analyzed by the same equipment configuration and the resulted spectra are shown in figure 6. It shows the characteristic groups 'peaks for DEP, DBP and DOP in the samples.

The appearance of different types of plasticizers into the same sample was due to that the used plastic materials for the manufacture of these toys, were taken from recycled plastics. The samples B, D and E were verified to contain PVC, while samples A and C show no evidence for the type of their plastic material.

Conclusion

Many toys and children products sold in the Egyptian market contain phthalates levels exceeds the authorized limits. The samples examined in this work do not cover the entire children products, so further investigation is needed.

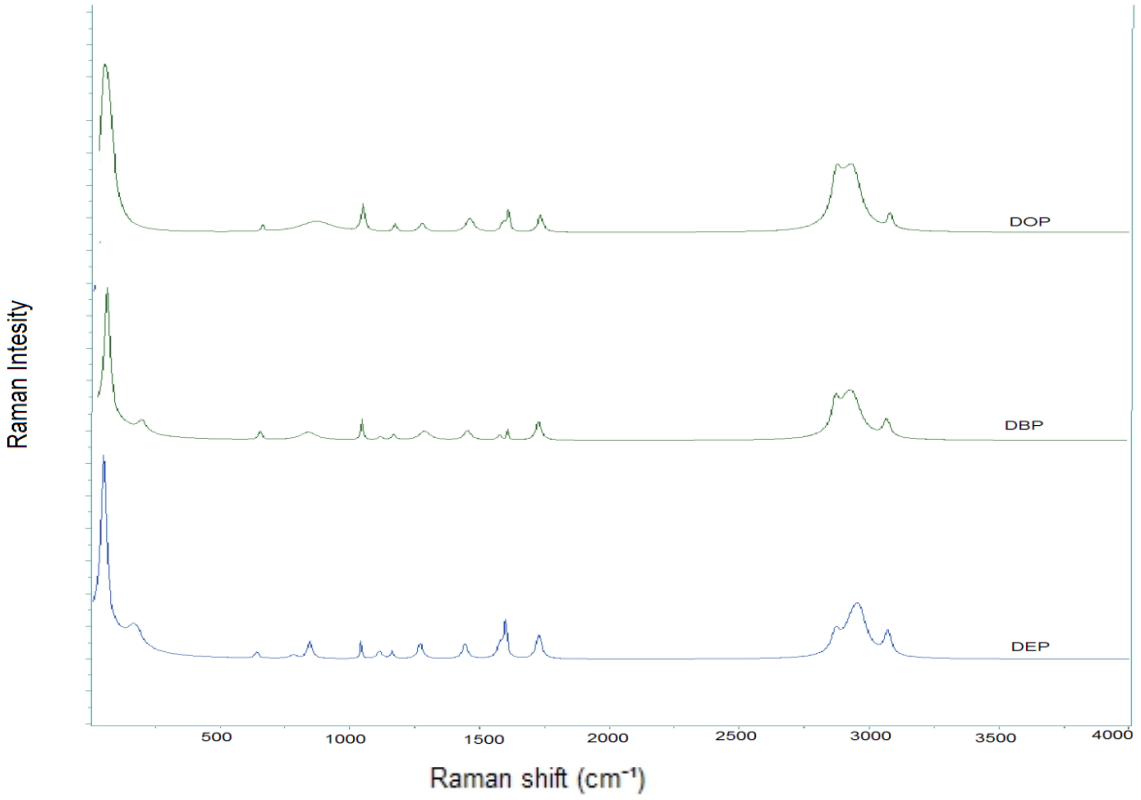
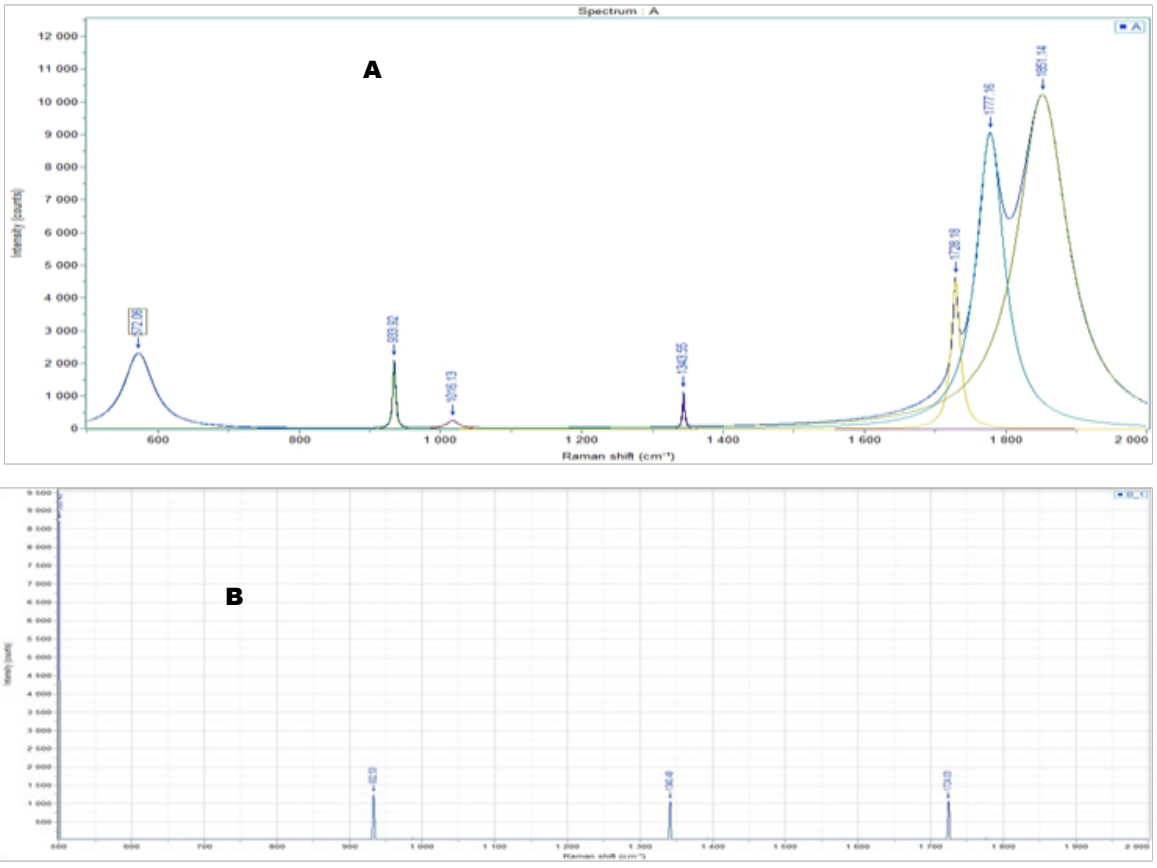


Fig.5. Raman spectra for phthalate standards.



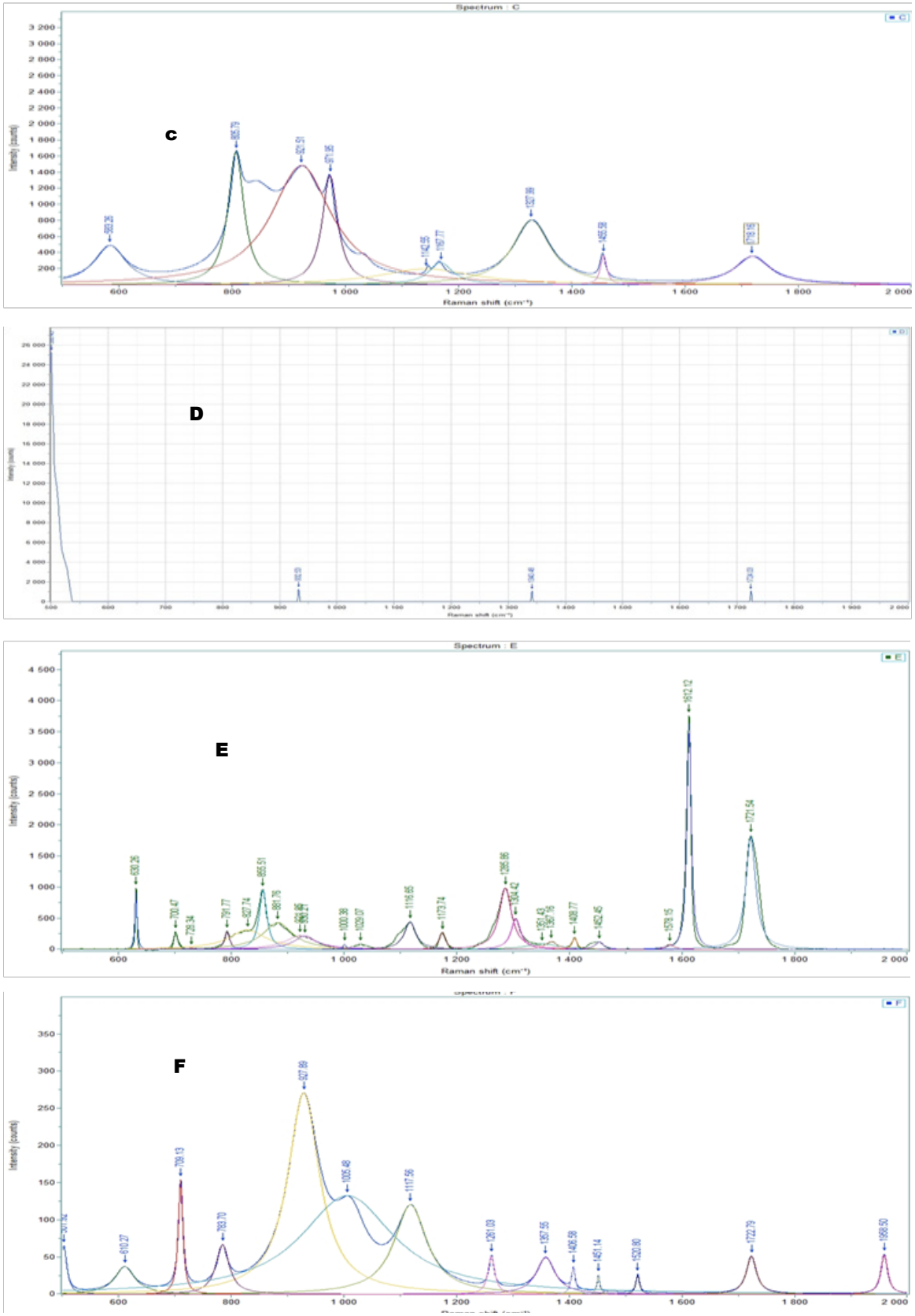


Fig.6. Raman spectra for toys samples: (A) Plastic tractor; (B)rattles toy; (C) plastic horse carriage; (D) Whistle; (E) Water bottle and (F) baby sucker.

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تقييم مستوي بعض الفيثالات في لعب الأطفال المتداولة في السوق المصري

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في هذا البحث تم فحص نسبة ثلاثة أنواع من الفثالات الموجودة في لعب ومنتجات الأطفال التي يتم تداولها في السوق المصري وهي ثنائي ايثيل فيثالات وثنائي بيوتيل فيثالات وثنائي اوكتيل فثالات . تم استخلاص الفثالات من العينات باستخدام ثنائي كلوروميثان وبعد ذلك تم عمل تنقية . تم استخدام جهاز الكروماتوجراف السائل فائق الاداء المزود بمستشعر الاشعة الفوق بنفسجية وقد أظهرت النتائج أن النسب الموجودة في العينات أعلي من المصرح بها حيث كانت نسب ثنائي ايثيل الفثالات تتراوح بين (0.65-0.002 %) وذلك في 65% من العينات ، فيما تراوحت نسب ثنائي بيوتيل فثالات بين (0.93%-0.008) وذلك في 60% من العينات ، بينما تراوحت نسب ثنائي أوكتيل فثالات بين (1.15%-0.02) وذلك في 90% من العينات ، ولتأكيد هذه النتائج تم فحص العينات باستخدام جهاز مطياف الرامان.